AN ABSTRACT OF THE THESIS OF

Maqsood Hassan Qureshi for the degree of Doctor of Philosophy in Soil Science presented on April 6, 1999. Title: NITROGEN AVAILABLE TO WINTER WHEAT AS INFLUENCED BY PREVIOUS CROP IN A MOIST XERIC ENVIRONMENT

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Neil W. Christensen

Rotating wheat with other crops is a common practice in the Willamette Valley of western Oregon. Depending upon previous crop and soil type, current N fertilizer recommendations for wheat in the Willamette Valley vary widely. Excessive fertilizer poses environmental risk, whereas lower N inputs than required by the crop represent economic losses to growers. Growers and their advisors face the challenge to minimize the environmental risk, and at the same time to maintain or increase economic returns. Questions are often raised concerning the efficient use of N fertilizer and accurately predicting the amount of N needed by wheat following different crops.

The first study measured growth, N uptake and N use efficiency (NUE) of winter wheat grown after either a legume or oat for three years. In all three growing seasons, winter wheat showed higher biomass, N uptake and NUE when grown after a legume than after oat. The contribution of legume was evident before the wheat was fertilized in spring, indicating that legume N had mineralized in fall or winter.

Contribution of soil N to wheat suggested that fertilizer N can be reduced by 44 kg N ha^{-1} if a legume is grown previously. Nitrogen use efficiency estimated 50 to 70 days after N application by isotopic method (24 to 94 %) was comparable with that estimated simply by difference (21 to 94 %) at the same time.

The second study predicted gross mineralization rates using analytical models. Comparable N mineralization was predicted by a model assuming remineralization and a model assuming no remineralization, suggesting that remineralization was negligible. In the spring, mineralization-immobilization turnover was at a lower pace than expected in both rotations. In two growing seasons, gross mineralization rates were higher where the previous crop was legume (0.37 to 0.74 kg^{-1} ha^{-1} day^{-1}) as compared to where oat was grown previously (0.14 to 0.6 kg^{-1} ha^{-1} day^{-1}). Negative net mineralization indicated that fertilizer N was immobilized in the oat-wheat rotation.

The third study evaluated calibration and digestion techniques used to determine elemental concentration in grasses. Use of a dry ashed standard to calibrate the ICP spectrometer generated highly variable calibration curves and was not a viable calibration method. Good agreement was found between chemical and microwave digested standards. Dry ashing resulted in considerable S and Mn losses, whereas, perchloric acid digestion and microwave digestion showed similar results. Our study suggests that if routine analysis are to be performed for macro nutrients or involve trace level work, the best method is microwave digestion with chemical standard calibration of ICP spectrometer.

NITROGEN AVAILABLE TO WINTER WHEAT AS INFLUENCED BY PREVIOUS CROP IN A MOIST XERIC ENVIRONMENT

by

Maqsood Hassan Qureshi

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APPROVED:

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Major Professor, representing Soil Science.

Redacted for privacy

Chair of Department of Crop and Soil Science

Redacted for privacy

Dean of Graduate School

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THIS THESIS IS

DEDICATED TO MY MOTHER

SAEEDA QURESHI

NITROGEN AVAILABLE TO WINTER WHEAT AS INFLUENCED BY PREVIOUS CROP IN A MOIST XERIC ENVIRONMENT

CHAPTER 1

INTRODUCTION

Study I

Nitrogen is the most common limiting nutrient in wheat production. Wheat is grown after different crops on a variety of soils in the Willamette Valley of western Oregon. Nitrogen fertilizer requirements for winter wheat in the Willamette Valley vary with soil type and previous crop. Knowledge about the optimum amount of fertilizer N for wheat is important to avoid economic losses as well as to reduce environmental risks. Both of these issues are related to the N use efficiency of wheat.

Traditionally, N use efficiency is estimated by the "difference method" in which the total N uptake of the above ground biomass of fertilized plot minus the total N uptake of N in unfertilized plots is expressed is a percentage of the amount of fertilizer applied (Dilz, 1987, Rao et al., 1991). Similarly, if multiple N rates are used, the N use efficiency can be estimated as the slope of regression line when N uptake is regressed over the amount of N applied (Terman and Brown, 1968). However, these approaches assume that the both fertilized and control plots are similar with respect to nitrogen transformation processes, which may be not true because of the priming effect of added N (Westerman and Kurtz, 1974). Nitrogen use efficiency can also be estimated by isotopic dilution, which involves adding nitrogen enriched or depleted in ^{15}N to the soil plant system and following the extent to which the ^{15}N has interacted with the system (Hauck

and Bremner, 1976). The isotopic dilution method of estimating nitrogen use efficiency does not require a control, and provides more accurate estimate than the conventional by difference method.

It has been shown that nitrogen use efficiency is influenced by crop management factors including timing, source of fertilizer N (Christensen and Meints, 1982) and crop rotation (Baldock et al., 1981). Among these factors, crop rotation is of paramount importance since it provides some or all of the N requirement of succeeding crop if a legume is included in rotation (Varvel and Peterson, 1990). Crop rotation also affects plant N availability by influencing the soil N immobilization and mineralization process (Pierce and Rice, 1988). In this context a long term study was initiated in 1995 at the Hyslop field laboratory with the objectives:

- To determine the long-term effect of previous crop on growth and nitrogen uptake of winter wheat.
- To evaluate if the previous crop affects nitrogen use efficiency of winter wheat.
- To compare different methods of estimating nitrogen use efficiency (NUE).
- To predict the amount of N fertilizer needed to wheat following a legume or oat.

Two rotations, winter wheat following a legume or winter wheat following oat, were established for three growing seasons. In the winter of each growing season, five nitrogen treatments ranging from 0 to 200 kg N ha^{-1} were established in each rotation. Four micro plots were established in the 100 kg N ha^{-1} treatment of each rotation. The microplots were fertilized with labeled ammonium or labeled nitrate at the same rate as their relative large plots. Plant and soil samples were collected at fertilization and at the

time wheat had accumulated about 1500 growing degree days (GDD) from micro plots and at maturity from large plots. Nitrogen use efficiency was estimated by isotopic method and by difference from micro plots data and by least square and simple by difference method from the large plots data.

Results showed:

- Over three growing cycles wheat after legume showed higher biomass, N-uptake and NUE, than wheat after oat.
- The lower NUE in wheat following oat may be a result of immobilization of applied N or the physiological incapability of wheat to utilize the applied N.
- Both isotopic method and simple by difference methods showed comparable results in micro plots, indicating that the N pool substitution was minimal over the experimental interval.
- The least square and by difference methods showed good agreement, but the least square method was more precise, and is a better choice if multiple rates are used.
- The effect of legume was evident as early as Feekes GS 4, indicating that legume N had mineralized in fall or winter.
- Contribution of legume N to winter wheat showed that fertilizer rates can be reduced by 44 kg N ha^{-1} without any yield loss.
- If the wheat is grown after a non-legume, fall N application as a starter fertilizer is necessary to alleviate N deficiency that may limit subsequent crop response to applied N.

Study II

Nitrogen mineralization is transformation of organic N into mineral form; the reversal of mineralization is immobilization. Mineralization and immobilization occur in soil simultaneously (Jansson and Persson, 1982). Both of these process are complex and therefore difficult to accurately predict (Powlson and Barraclough, 1993). There have been numerous approaches to measure N mineralization, however, most of the efforts were directed to measure net mineralization, because the absence of suitable methods to estimate gross mineralization. The concept of measuring gross mineralization using labeled N was pioneered by Hiltbolt et al. (1950). Later Kirkham and Bartholamew (1954) developed an analytical model of zero order to estimate gross N mineralization rates by measuring the rate of dilution of the enriched ^{15}N pool. Since the development of analytical models, most of the research was directed to measure gross rates in laboratory, while field measurement of gross rates remained scarce because of cost of labeled N and spatial variability in the field (Gaunt et al., 1998). Field measurements of N transformation rates could potentially improve fertilizer N recommendations by more accurately predicting the amount of fertilizer required by a crop. A field study was conducted to evaluate the impact of previous crop on nitrogen transformation rates. The objective of the study were:

- To compare the effect of previous crop on gross mineralization rates.
- To compare different approaches to estimate N transformation rates
- To evaluate the contribution of legume to winter wheat grown in the Willamette Valley.

The experimental design was the same as that of study I. Three analytical models based on different assumptions were used to estimate gross N mineralization and nitrification rates. Model I assumes that immobilized labeled N does not remineralize during the course of experiment, whereas, Model II corrects for the assumption of remineralization. Model III, which is similar to model I, utilizes mean pool abundance of ^{15}N in the mineral pool for estimated from plant uptake of labeled N.

Results showed that:

- Models considering remineralization and no remineralization of labeled N estimated comparable N transformation rates, indicating that mineralization - immobilization turnover (MIT) was slow in late winter and early spring.
- Mineralization rates were higher in wheat following legume as compared to wheat following oat.
- The effect of previous crop was more pronounced in the second year as compared to the first year.
- Fertilizer N was immobilized in wheat following oat in both growing seasons.
- Contribution of legume N was small in early spring, indicating that most of the legume N had mineralized early in the season.
- Even though the effect of legume was evident, winter wheat in the Willamette Valley may not fully benefit from legume N because of early mineralization and subsequent nitrification and leaching.

Study III

Elemental analysis of plant tissue is an integral part of today's modern soil fertility programs (Wolf, 1982). Plant tissue analysis involves destruction of organic matter using heat, acid or bases (Burguera and Burguera, 1998), and is characterized into two main procedures: dry ashing and wet acid digestion (Jones and Case, 1990). Dry ashing involves heating plant samples at high temperature in a furnace to combust organic matter and is simpler than wet ashing. Wet ashing involves heating plant tissue after treating with different acids, usually H_2SO_4, HNO_3 and $HClO_4$, either separately or in combination (Jones and Case, 1990). Both of these methods are subject to limitations. Dry ashing may result in losses of some easily volatile elements like Fe, Cu, B, Zn. (Nikdel and Temelli, 1987; Schnug and Haneklaus, 1996). Similarly, wet digestion requires special equipment, and may result coprecipitation of elements being analyzed (Greenberg et al., 1990). Another limitation of these procedures is that they require several hours, and if wet digestion is being performed a constant supervision is required. Microwave digestion hastens the digestion process and volatilization is minimal because it is performed in closed vessels (Kingston and Jassie, 1986). However, to select one digestion method over another has been a matter of controversy, and also the efficacy of any of these methods is dependent on the plant matrix. Moreover, most of the work to evaluate a digestion method has been directed to horticultural plants and very few studies have compared these techniques for forage analysis. This study was carried out with the objectives:

- To evaluate the effect of different standards commonly used to calibrate the ICP on the determination of elemental concentration of forages.
- To compare dry ashing, conventional wet ashing and microwave assisted wet ashing as applied to forage tissue.
- To adopt and recommend a technique which is best suited for determining elemental concentration of forage tissue.

Tall fescue samples were collected from research plots at six different dates in 1993. The samples were digested using three digestion method: 1) dry ashing, 2) microwave acid digestion and 3) Perchloric acid digestion. The dry ashed and microwave digested samples were analyzed on ICP, which was calibrated either with a synthetic standard, dry ashed standard, or microwave digested standard. The perchloric acid digested tissue were analyzed on AAS.

Results of the study showed that:

- Using a dry ashed standard is not a viable method for ICP calibration.
- Both microwave digested standard and chemical standard yielded comparable results, indicating that microwave digested standard is as reliable as a chemical standard.
- If the purpose of analysis is to make fertilizer recommendations for major elements, dry ashing can successfully be used.
- Dry ashing should be avoided, if the analysis involves trace level work.
- Both perchloric acid digestion and microwave digestion are equally effective for determining elemental concentration of grasses, however, the explosion hazards limit use of perchloric acid.

- The best method for simultaneous determination of a variety of elements in grass tissue is microwave assisted digestion with chemical standard calibration.

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CHAPTER 2

GROWTH, NITROGEN UPTAKE AND N USE EFFICIENCY OF WINTER WHEAT GROWN AFTER A LEGUME OR OAT

INTRODUCTION

Wheat is among the top five agricultural commodities in Oregon. During 1996 about 83,000 acres of wheat were harvested in the Willamette valley of western Oregon. Wheat is often rotated with other crops to diversify cropping systems, to break weed and disease cycles, and to replenish the soil with nitrogen fixed by legumes. Current nitrogen fertilizer recommendations for winter wheat grown in western Oregon are based on previous research (Hart et al., 1992). Recommendations for nitrogen fertilizer vary from 67 to 200 kg N ha^{-1} across all rotations and consider both the previous crop and soil type (Sebastian, 1995). Wheat growers and agribusiness field representatives often observe great differences in wheat growth and grain yield response to N fertilizer from field to field, across and within rotations. Questions are often raised concerning the quantity of N fertilizer needed for wheat in different rotations. Specific goals for growers are to maintain or increase grain production while simultaneously reducing environmental risk from excessive nitrogen fertilizer application. Traditionally, modern cropping systems depend on inputs of commercial fertilizers to maintain or increase crop yields (McKenny et al., 1993). In some cases N fertilizer is applied in excess of the amount required for optimum yield and is not utilized efficiently by the crop plants (Angel et al., 1993; Prunty and Montgomery, 1991). Generally only about 50 to 60 % of applied N is taken up by plants; about 25 % is immobilized by soil microrganisms (Hauck, 1984). The remainder

of the applied N (≈ 25 %) leaves the soil-plant system by leaching of nitrate or gaseous losses through denitrification or ammonium volatilization. In a study conducted in the Willamette valley of western Oregon, Kjelgren (1984) found that winter wheat uptake of applied N ranged between 42 to 67 % and about 10 to 31 % of applied the N was lost from the soil-plant system after harvest. In a recent study of intensive wheat systems in Mexico, Matson et al. (1998) found that conventional fertilizer practices resulted in a loss of 70 kg ha^{-1} of applied N through excessive denitrification caused by water-logged conditions due to irrigation. These losses of N represent economic loss to the growers and have also become an environmental concern because of increased nitrate contamination of the ground water and enhanced nitrous oxide emissions into the atmosphere.

To alleviate the adverse environmental impacts of nitrogen and reduce input losses, an increased emphasis on improving N use efficiency in field crops exists. This effort involves 1) adopting efficient management strategies (Hargrove, et al., 1988; Fiez et al., 1995), 2) development of cropping systems which influence N use efficiency by affecting soil and plant physiological processes. The processes considered for increased NUE are associated with N absorption, translocation, redistribution and assimilation (Moll et al., 1981).

The phrase *N use efficiency* refers to relationships between: (i) yield and the amount of N applied, which is termed as yield efficiency; (ii) yield and N uptake, which is physiological efficiency; and (iii) N uptake and amount of N applied, the recovery efficiency. On the cumulative basis, these relationships can be shown by the following

expressions:

$$Yield\ efficiency = \frac{Y_i - Y_0}{N_i} \quad [1]$$

$$Physiological\ efficiency = \frac{Y_i - Y_0}{NR_i - NR_0} \quad [2]$$

$$Recovery\ efficiency = \frac{NR_i - NR_0}{N_i} \quad [3]$$

Where:

Y_i and Y_0 are yields of fertilized and unfertilized plots respectively.

N_i is the amount of nitrogen applied.

NR_i and NR_0 are the nitrogen uptake of fertilized and unfertilized plots, respectively.

From the above expressions one can find that yield efficiency is the product of physiological efficiency and recovery efficiency. However, yield efficiency has little merit for comparisons of N management systems and cropping situations. This is because most of the time yield response curves, as a function of N rate, are curvilinear and yield efficiency varies with the shape and the portion of the response curve used for calculation. Physiological efficiency is analogous to yield efficiency when N uptake is substituted for N rate in equation [2]. Physiological efficiency represents the theoretical

attainable yield efficiency when N recovery efficiency is = 1 (Bock, 1984). The primary limitation in estimating physiological efficiency is that 100 % N recovery efficiency is seldom achieved under field conditions (Tomar and Soper, 1981). Due to ammonia volatilization, nitrate leaching and immobilization of fertilizer N, nitrogen recovery efficiency generally averages about 50 % (Allison, 1966; Christensen and Killorn, 1981, and Soper et al., 1971). The most widely used definition of nitrogen use efficiency is the recovery efficiency. In general, more emphasis is placed on technology and practices for altering N recovery efficiency than for altering physiological efficiency as a means of improving yield efficiency (Bock, 1984). Enhancing N recovery efficiency not only improves economics of N fertilizer but also reduces the potential for adverse environmental impact. In this study, N recovery efficiency is also referred to as N use efficiency.

The traditional approach for estimating N use efficiency is the "by difference method" as shown by equation [3] (Rao et al., 1991). The by difference method assumes that the both fertilized and unfertilized plots behave similarly in terms of N mineralization, N immobilization and other dynamic N processes in the soil (Westerman and Kurtz, 1974). However, this assumption is often not completely fulfilled because increased root growth (Bock, 1984; Olson and Swallow, 1984) and/or stimulated microbial activity (Westerman and Kurtz, 1973) in the fertilized plots often results in higher N use efficiency than in unfertilized plots.

Linear regression can also be used to calculate N use efficiency by difference. The regression approach is based on the fact that N uptake for wheat is linear over rather

broad ranges of applied N. In a study conducted by Kelly (1995) in Kansas, the relationship between winter wheat N uptake and fertilizer N in three different rotations was linear to 135 kg N ha^{-1}. Similar results were observed by Kjelgren (1985) in western Oregon, where the N uptake of winter wheat was linear to 200 kg N ha^{-1}. The regression approach involves fitting a simple linear regression model, (equation [4]) to the crop nitrogen uptake as a function of N rate, and estimating the intercept and the slope of the regression line.

The intercept, "NR_0", is an estimate of the N uptake of the crop when no N is applied. The slope, "b", is the estimate of N use efficiency (Hauck and Bremner, 1976) and N_i is the amount of nitrogen fertilizer added. The limitation of the regression approach is that it can only be used with multiple N rates.

$$Y = NR_0 + bN_i \qquad [4]$$

Terman and Brown (1968) have classified N uptake responses into three categories based on the magnitude of observed uptake of check plot (nil N) measured directly or estimated by linear regression. In the "Type one" response, the observed N uptake of check plots is lower than that estimated by regression, and the estimated percent N use efficiency decreases with an increase in applied N. This response suggests that crop has utilized proportionally more native soil N in the presence of applied N than in its absence. In the "Type two" N response curve, the observed N uptake of the check is higher than estimated by regression, and the N use efficiency estimated by difference

increases with amount of applied N. This response is a result of immobilization or fixation of initial increments of added N. In the "Type three" response, estimated and observed N uptake of check plots are similar. The N use efficiency does not change with fertilizer increment and the estimated recovery by difference between fertilized and unfertilized is same as that estimated by regression. They suggested that N use efficiencies estimated by difference method with either isotopically labeled or unlabeled N are subject to limitation by immobilization and interchange in the soil and that isotopic labeling technique has little advantage over other methods if multiple rates are compared. As by difference methods cannot distinguish between N furnished by the soil and N from fertilizer, only apparent N use efficiency is estimated by the difference method. However, apparent N use efficiency is a useful parameter in studies of nitrogen balance and efficiency of N use in cropping systems (Dilz, 1987).

The most accurate method for estimating N use efficiency is by using stable isotopes of N. The use of stable nitrogen isotopes as a tracer is based on the fact that ^{14}N and ^{15}N occur naturally in an almost constant ratio of 272:1. Addition of a material to the soil plant system with an unusually high or low concentration of ^{15}N will result in an increase or decrease in the ^{14}N to ^{15}N ratio of the soil-plant system. The amount of change from the background level permits calculation of the extent to which the tracer has interacted with or become part of the system (Hauck and Bremner, 1976). The calculation of N use efficiency by isotopic dilution involves addition of enriched or depleted ^{15}N fertilizer and direct measurement of ^{15}N labeled fertilizer taken up by the plant, as calculated using equation [5]. Equation five is used for both cases, enriched ^{15}N

or depleted ^{15}N, however, in case of depleted ^{15}N the order of (A-C) and (B-C) is reversed (Hauck and Bremner, 1976).

$$\%N\ efficiency = \frac{NR_i \times (A - C)}{N_i \times (B - C)} \times 100 \qquad [5]$$

Where:

NR_i = nitrogen taken up by plants (kg N ha^{-1}).

A = atom % ^{15}N in the plant tissue which received ^{15}N.

C = atom % ^{15}N in the plant tissue which received no N {natural abundance (0.367) or measured value (i.e. 0.372 this experiment)}.

N_i = Amount of nitrogen fertilizer added (kg N ha^{-1}).

B = atom % in the fertilizer.

The isotopic tracer approach of estimating N use efficiency requires no control treatments, thus obviating the need to make assumptions as discussed earlier regarding the similarity of N transformation processes in fertilized and unfertilized plots (Hauck and Bremner, 1976). Also, contrary to the regression approach, it does not require multiple N rates. Although during the course of study, interchange between fertilizer N and soil organic N may occur, the major benefit from using isotopic labeling is that it provides the most accurate measure of the relative contributions of soil N and fertilizer N to plant uptake (Nielsen, et al., 1987).

Use of the isotopic tracer technique requires certain assumption be made. The major assumptions are: (i) isotope compositions in tracers are constant; (ii) living organisms cannot discriminate one isotope from another of the same element; and (iii) chemical identities of isotopes are maintained in biochemical systems. According to Hauck and Bremner (1976), these assumptions are valid for most studies in which ^{15}N compounds are used.

The estimated N use efficiency values calculated by the three approaches discussed above usually do not coincide with each other (Torbert et al., 1992). Generally the by difference methods estimates higher N use efficiency values than the isotopic dilution method. Low and Piper (1957), in a rye grass study, found that the N use efficiency calculated by difference was 9.5 % greater than that calculated by using labeled ammonium sulfate and urea. Westerman and Kurtz (1974) compared N use efficiency of sudan grass estimated by difference method and by isotopic dilution. They found that the by difference method over-estimated the N use efficiency of urea and oxamide N by 35 to 23 % and 31 to 35 %, respectively, when compared to the isotopic tracer method. Terman and Brown (1968) compared N use efficiency calculated by linear regression with that calculated by isotopic dilution. They concluded that the by difference method is oversimplified and does not effectively characterize the N use efficiency of applied fertilizer. Similar results have been observed by Hauck (1971) in a comparison of N use efficiency calculated by difference and by labeled N.

Differences in N use efficiency have been reported for N application methods (Mahli and Nyborg, 1985; and Sower et al., 1994), timing of application and source of N

fertilizer, (Christensen and Meints, 1982; and Alcoz et al., 1993) and crop rotation (Baldock et al., 1981; Hesterman et al., 1987). Among these management factors, crop rotation is considered to be of increasing importance because of its potential to reduce N fertilizer needs if a legume is included in the rotation (Varvel and Peterson, 1990). Crop rotation refers to a system of a specific crop sequence in which the succession of crop is repeated (Yate, 1954). Crop rotations and their benefits to agriculture have long been known. The scientific and popular literature provide a number of explanations for the general observation of higher yields when crops are grown in rotation as compared to monoculture.

Crop rotation influences N transformation and losses from soil by altering various soil N sources and pools in terms of both quantity and availability to plants. The N sources and pools which are mainly influenced by rotation are (i) crop residue N; (ii) soil inorganic N; (iii) symbiotically fixed N, (iv) microbial biomass N; and (v) organic N (Pierce and Rice, 1988).

Difference in composition of residue from previous crops influences the amount and rate of N mineralization from crop residue. In a study conducted in Argentina on wheat grown in rotation with soybean, sunflower and maize with residue incorporated, Echeverria et al. (1992) found that wheat after maize produced the lowest yield and was more responsive to N fertilization than wheat grown after soybean or sunflower. They suggested that the residue of the previous crop generated different soil N availabilities which affected subsequent wheat yields. Kelly (1995) observed that the grain yield of winter wheat after three years of rotation with oat, soybean and grain sorghum was higher

when the wheat followed oats than following either of the other crops. He also observed that significant amounts of mineralizable N were available to wheat when the previous crop was either oats or soybean, whereas, when wheat followed sorghum, the amount of residual N was considerably lower. The higher mineralizable N of an oat-wheat rotation was because this rotation was established on a site which had been under native grass production. He suggested that the differences between yield and N response were largely due to influence of grass on mineralization of soil N or immobilization of applied N.

Inorganic N accumulated as nitrate in the soil is affected by the frequency of crops receiving N above that required for maximum yield. Roth and Fox (1990) found that soil nitrate accumulation to a depth of 120-cm in continuous corn ranged from 41 to 138 kg NO_3-N ha^{-1}. A direct relationship was observed by Olsen et al. (1970) between the total NO_3 in the soil profile and the frequency of corn and applied nitrogen in rotation. These data indicate that continuous corn would leave more NO_3 in the soil profile than when corn is grown in rotation with other crops. The increased amount of residual NO_3 may have negative effects if moved to the groundwater before being utilized by the subsequent crop. Also, high concentration of inorganic N in the soil may reduce the ability of a succeeding legume to fix atmospheric N.

Symbiotically fixed N_2 is influenced by rotation in two ways. First, the rotation may affect the survival of N_2 fixing organisms by influencing nutrient status and the pH of the soil. Hiltbold et al. (1985), in a study of a cotton-corn-soybean rotation, observed that a rapid decline in *R. japonicum* population occurred in the year cotton was growing following soybean, and the decline was more pronounced when the soil was not amended

with P, K and lime. Soybean symbiont N_2 fixers were only able to survive the intervening years in sufficient populations to infest the next soybean crop when the soil was limed and P and K were added. Second, the rotation affects the symbiotic N_2 fixation by utilizing residual inorganic N from the previous crop. Bezdicek et al. (1974) reported a decrease in nodule mass and N_2 fixation rate with the addition of inorganic N fertilizer. When N fertilizer was added to the preceding rye crop, the rye removed sufficient N to result in an increase in N_2 fixation and soybean grain yield.

Microbial biomass comprises an active soil N pool. The turnover of the microbial pool is a potential source of plant available N. Bolton et al. (1985) found that the microbial C and N were significantly higher in a winter wheat-winter pea-spring pea rotation than in a winter wheat-spring pea rotation. An indirect measure of microbial biomass is activity of selected enzymes. Dick (1984) reported a significantly higher activity of phosphatase, urease, and amidase in corn-oat-alfalfa rotation than in corn-corn and corn-soybean rotations.

The organic soil N pool is the most studied pool within the context of rotation. Rotation has been found to increase the soil organic N or reduce the losses of N from the organic pool. Unger (1968) reported that total organic soil N content was higher in a wheat-wheat rotation as compared to a wheat-fallow rotation. Another parameter for characterizing the soil organic N pool is mineralizable N. In a long-term rotation study involving continuous wheat, wheat-fallow and wheat-alfalfa, Janzen (1987) found that the mineralizable N was significantly higher in continuous wheat than in the other two rotations.

Legumes are generally grown in rotation because they are often credited with supplying large amounts of N to the succeeding nonleguminous crops (Hesterman et al., 1987). Legumes can also act as a catch crop for soil inorganic nitrogen, thus conserving the soil N and reducing leaching and denitrification (Walters et al., 1992). The potential N contribution from a legume crop to the succeeding crop is substantial. Estimates of fertilizer N value of alfalfa to a following corn crop have been as high as 180 kg N ha^{-1} (Baldock and Musgrave, 1980). However, additional beneficial effects of legumes to succeeding crops have also been reported. These effects may include reduced disease infestation, improved soil physical properties, and added growth promoting substance in the legume residue (Barber, 1972; Page and Willard, 1946; and Ries et al., 1977). Thus, the total effect of a legume on subsequent crop yield may be divided into two categories: (i) the effect of N supplied by legumes; and (ii) the net effect of all contributions when N in not limiting (Baldock and Musgrave, 1980). Knowing the contribution of these effects plus the N use efficiency of the subsequent crop may allow N fertilization to be optimized for decreased inputs and reduced N losses due to leaching and denitrification.

The purchase of N fertilizer represents 20 % of the total variable costs of producing wheat in the western Oregon (Taylor et al., 1990). Maximizing N use efficiency will increase the growers profit, maintain or increase crop yield and quality, and minimize the risk of undesirable effects in the environment which result when N is used inefficiently.

In this context, a long-term experiment was initiated in 1995 at the Hyslop field laboratory operated by the Department of Crop and Soil Science at Oregon State

University. The experiment consists of legume-winter wheat and oats-winter wheat rotations in which wheat receives one of five nitrogen treatments ranging from 0 to 200 kg N ha^{-1}. The objectives of this study were to: (i) determine the long-term effect of previous crops on growth and N uptake of winter wheat; (ii) evaluate if the previous crop affects N use efficiency of winter wheat; and (iii) compare different methods of estimating N use efficiency. The results of the first three years of this experiment are presented in this study.

MATERIALS AND METHODS

A long-term field experiment was initiated in the fall of 1994 at Hyslop Field Laboratory near Corvallis, OR. The soil on the farm is a Woodburn silt loam (Fine-silty mixed, mesic, Aquultic Argixerolls), a moderately well drained soil containing 113 mg kg^{-1} of Bray P, 208 mg kg^{-1} of NH_4OAc extractable K, 9.4 cmole(+) kg^{-1} of Ca, 0.7 cmole(+) kg^{-1} of Mg, and a pH of 6.0 in the surface 0.1 m.

A 96-m by 45-m experimental area was selected in a field which had been fallow in 1993-94. Crimson clover (*Trifolium incarnatum* L.) was planted over the entire area in fall 1994. In spring 1995 the area was subdivided into 6-m by 45-m plots. Two plots in each of four replications were fallowed, one was planted to oats (*Avena sativa* L.), and one was left in clover (Fig. 2-1).

This crop sequence was the same in 1995 and 1997. However, due to the slug (*Arion hortensis* F.) damage, winter peas (*Pisum sativum* L.) were replanted before wheat in 1995-96. The established cropping sequence consisted of two rotations: (i) a legume (crimson clover or pea) followed by wheat (*Triticum aestivum* L.); and (ii) oats followed by wheat.

In the fall, oats and legume crop residue was incorporated and 'Stephens' soft white winter wheat was planted in each strip. Planting dates for each year are shown in (Table 2-1). Wheat was planted at the rate of 30 seeds 1000 cm^{-2} using a double disc drill having 18-cm row spacing. Each 6-m by 45-m main plot consisted four drill passes of six rows each. Phosphorus at a rate of 10 kg ha^{-1} was banded with seed each year. Because

of the poor stand of wheat following oats during 1995-96 and 1996-97 growing seasons, 20 kg N ha^{-1} was also banded with seed in 1997-98. The N was banded in such a way that one row in each drill pass remained unfertilized. When cropped to wheat, each of the main plots was divided into five 6-m by 9-m sub-plots to establish five N fertilizer treatments ranging from 0 to 200 kg N ha^{-1} in 50 kg N ha^{-1} increments. Nitrogen fertilizer treatments were applied as urea [$CO(NH_2)_2$] using a manual drop spreader during the second half of February 1997 and 1998. In 1996, excessive rains delayed nitrogen fertilizer application until mid-March (Table 2-1). While nitrogen treatments were randomly assigned to each of the sub-plots, the rate of N remained the same whenever wheat was grown on that sub-plot. Oats plots were fertilized in June of each year at a rate of 66 kg N ha^{-1}, whereas clover or pea plots were not fertilized.

The arrangement of plots was a randomized complete block design split plot with four replications. Treatment variables included rotation (2) as main plot and N treatments (5) as sub-plots, resulting in a 2 x 5 x 4 factorial (Fig-2-1). Each year paired plots were rotated between legume or oats and wheat in each of the four replications.

Plant tissue samples from the large plots were collected at the time of fertilization (Feekes GS 5), and at maturity (Feekes GS 11.4) every year. At the time of fertilization, samples were taken by randomly selecting and clipping a portion of 1- meter from a single row. Pre-harvest tissue samples were taken by clipping 1.5-m of four different rows at randomly selected locations. All four sub-samples were then composited to get a representative sample. During 1997 and 1998, additional plant tissue samples were collected about 25 days after fertilizer application from 0-N and 100-N plots. In 1998,

plant samples were also collected about 70 days after fertilizer application. Each tissue sample taken 25 or 70 days after fertilization was a composite of three sub-samples collected from 0.8-m of 2 adjacent rows at three different locations within an N rate sub-plot.

Soil samples were collected at the same time the tissue sampling was done. Pre-fertilization soil samples were taken from 0 to 30 and 30 to 60-cm depths and were a composite of three cores. Soil samples taken 25 days after fertilization were a composite of six cores which were randomly collected at 0-10 and 10-20 cm depths from 0-N and 100-N plots. An intensive preplant and postharvest soil sampling was also done at five depths ranging from 30 to 150 cm from the 0, 150 and 200 kg N ha^{-1} treatments.

Grain yield from large plots was obtained from pre-selected drill passes in each rotation. These selected drill passes had not been disrupted by plant tissue sampling. To avoid border effects, about 0.5-m of each end of N rate sub-plots was cut and discarded. Harvesting was done with a small-plot combine. Seed was collected into bags, cleaned on a Pelz rub-bar cleaner and then weighed to determine grain yield.

Four micro plots were established within the 100 kg N ha^{-1} treatments in each rotation by inserting open-ended galvanized sheet metal cylinders into the soil. Each cylinder had a radius of 45-cm, and was manually pushed into the soil to a depth of 20-cm. The micro plots were arranged to include two adjacent rows of wheat. One of the micro plots was fertilized with $^{15}NH_4NO_3$, and another was fertilized with $NH_4{}^{15}NO_3$, while the remaining two were left as unfertilized controls in 1996 and 1997. In 1998, all

four micro plots received labeled N fertilizer. Two micro plots received $^{15}NH_4NO_3$ and the other two received $NH_4{}^{15}NO_3$.

The microplot treatments consisted of application of 100 kg N ha^{-1} as a solution of $^{15}NH_4NO_3$ containing 6.811 atom % ^{15}N or $NH_4{}^{15}NO_3$ containing 5.163 atom % ^{15}N in 1996 and 1997, and 5.182 and 5.031 atom % ^{15}N, respectively, in 1998. The fertilizer solution was prepared in the lab by dissolving $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ granules into 250-mL plastic bottles in equivalent amounts to give 100 kg N ha^{-1}. At the time of application, the solution was diluted to 1 L in a 1.5 L plastic jar and sprinkled over the micro plots as uniformly as possible. Nitrogen fertilizer was applied to the microplot one day after the large plots were fertilized. The micro plots were covered with a plastic lid while fertilizing large plots.

Plant and soil samples were taken from micro plots at the time of fertilization and again after about 50 to 70 days of fertilizer application when the crop had accumulated about 1450 to 1615 growing degree days (GDD). The sampling time corresponded to Feekes GS 5 and 8-9 respectively. Plant samples were collected by clipping the rows inside the micro plots approximately 0.75-cm above the ground. In 1996 and 1997 the plant samples taken at the time of fertilization were from one of the unfertilized controls. The post-fertilization plant sampling included two fertilized micro plots, and the remaining control microplot. In 1998, the two additional fertilized micro plots were sampled after about 25 days of fertilization. Soil samples were collected from micro plots at the same time tissue samples were collected. The soil samples were composites

of ten cores collected at 0 to 10 and 10 to 20-cm depths by using a 2-cm soil sampling probe.

Plant samples were dried at 70 ^{0}C in a forced air oven, weighed and ground in Wiley mill to pass 1-mm mesh.. The plant samples were analyzed for total N and C concentration by Leco CNS 2000 combustion analyzer (Leco Corp., St. Joseph, MI) in the Central Analytical Laboratory of the Department of Crop and Soil Science, Oregon State University Corvallis, OR. The determination of C and N concentration by Leco involved introducing about 0.5 g of tissue into a combustion chamber. The furnace and oxygen in the chamber cause the sample to combust. The combustion process converts any elemental carbon and nitrogen into CO_2, N_2 and NO_x. These gases are swept by a carrier gas (helium) to the catalyst heater where NO_x gases are reduced into N_2. Finally these gases are passed through infrared red (IR) cell for determination of C concentration. After removal of CO_2 and H_2O in Lecosorb®/anhydrone tube, the gas is passed through a thermal conductivity (TC) cell for determination of N_2 concentration. For isotopic analysis, sub-samples from the ground tissue samples were finely ground (< 75 μm) in acid-washed glass jars, containing six to eight stainless steel bars, on a roller mill for 120 hours. Isotopic analysis for atom % ^{15}N were carried out by CN direct combustion isotope ratio mass spectrophotometer (Europia Scientific., Crewe, England) in the Stable Isotope Research Unit, (SIRU) of the Department of Crop and Soil Science, Oregon State University Corvallis, OR. The isotopic analysis involved introducing about 5-mg of sample into combustion chamber containing catalyst (CrO_3) granules. The combustion products CO_2, N_2, NO_x and H_2O are swept by a carrier gas (helium) into a tube containing

Cu wire at 600 ^{0}C, where NO_x species are reduced to N_2, followed by $Mg(ClO_4)$ and Carbosorb® traps for removal of H_2O and CO_2. The N_2 was purified by gas chromatography, and a small fraction of the effluent was admitted to the mass spectrometer via capillary tubing for measurement of *m/e* 28, 29 and 30, from which both total N and ^{15}N were determined.

Soil samples were air dried and ground to pass 2-mm sieve. Twenty grams of soil samples were extracted with 75-mL of 2M KCl. The extract was the filtered through Whatman No. 42 filter paper. An aliquot of 3.7-mL was pipetted into small plastic vials for the analysis of ammonium and nitrate nitrogen. The ammonium nitrogen concentration was determined by ALPKEM (Portland, OR) rapid flow autoanalyzer which complexes ammonium with salicylate to form indophenol blue. The color was intensified with sodium nitroprusside and measured at 660-nm. The nitrate nitrogen was determined using the same equipment by reducing nitrate to nitrite via a cadmium reactor and complexing the nitrite with sulfanilamide and N-(1-Napthyl)-ethylenediamine dihydrochloride to form red-purple color measured at 540-nm.

Climatological data for daily minimum and maximum air temperature, soil temperature at the depth of 10-cm, and daily precipitation from planting to the date of harvesting were obtained from Oregon Climate Service, Oregon State University, Corvallis, OR. Heat units (growing degree days (GDD)) were calculated by setting the base temperature = 0 ^{0}C, and averaging the daily minimum and maximum temperatures.

Biomass yield, nitrogen uptake and soil NH_4-N and NO_3-N concentration were statistically analyzed by standard ANOVA procedure for a randomized complete block

design, using MSTAT-C Ver 2.0. For biomass yield and nitrogen uptake, analysis of variance was used to test for the significant differences associated with rotation, nitrogen treatments, and their interactions. The same procedure was used for analyzing the ammonium and nitrogen concentration in the soil.

A logistic response model equation [6] was used to describe biomass accumulation and nitrogen uptake as a function of cumulative heat units. The parameters of the models describing total biomass accumulation and nitrogen uptake were estimated through non-linear regression by using STATGRAPHIC Ver 5.1.

$$N_h = \frac{K}{(1 + C\exp^{-rh})} \qquad [6]$$

Where:

N_h = Biomass yield or nitrogen uptake at heat unit h.

K = Maximum biomass yield or nitrogen uptake.

C = The ratio of the difference between maximum (K) and minimum (N_0) to the minimum biomass yield or nitrogen uptake, $C = (K\text{-}N_0)/N_0$.

r = Rate of increase (slope factor) per heat unit.

exp = The base of natural logarithm, 2.71828.

Nitrogen fertilizer use efficiency was calculated using the by difference method, which involved a) the difference of crop nitrogen uptake between 0 and 100 kg N ha^{-1} treatments divided by 100 (the amount of fertilizer added) equation [3], and b) the least

square method, which estimated the slope of regression line of crop nitrogen uptake as a function of N fertilizer added, equation [4]. Nitrogen uptake was regressed against all five N treatments ranging from 0 to 200 kg N ha^{-1}. Nitrogen fertilizer efficiency was also calculated from direct measurement of plant recovery of ^{15}N in the micro plots, equation [5]. Nitrogen use efficiency was calculated at the time of flag leaf emergence (Feekes GS 8-9) and at the time of maturity (Feekes GS 11.4). At flag leaf emergence the N use efficiency was calculated by recovery of ^{15}N, and by difference method from the micro plots data. In contrast, N use efficiency at maturity was calculated using only the two by difference methods.

Table 2-1. Dates, growth stages and growing degree days at planting, fertilization, and harvesting of winter wheat during 1995-96, 1996-97 and 1997-98 growing seasons.

	1995-96			1996-97			1997-98		
	Date	Feekes stage	GDD†	Date	Feekes stage	GDD	Date	Feekes stage	GDD
Planted	11/02/95	0	7	10/04/96	0	18	10/17/97	0	13
Fertilized									
Micro plots	03/17/96	4	971	02/23/97	4	1004	02/19/98	4	879
Plots	03/16/96	4	961	02/22/97	4	997	02/18/98	4	869
Harvested									
Micro plots	05/04/96	8	1455	05/02/97	9	1613	05/01/98	8	1509
Plots	07/25/96	11.4	2763	07/25/97	11.4	3019	08/04/98	11.4	3076

†Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C).

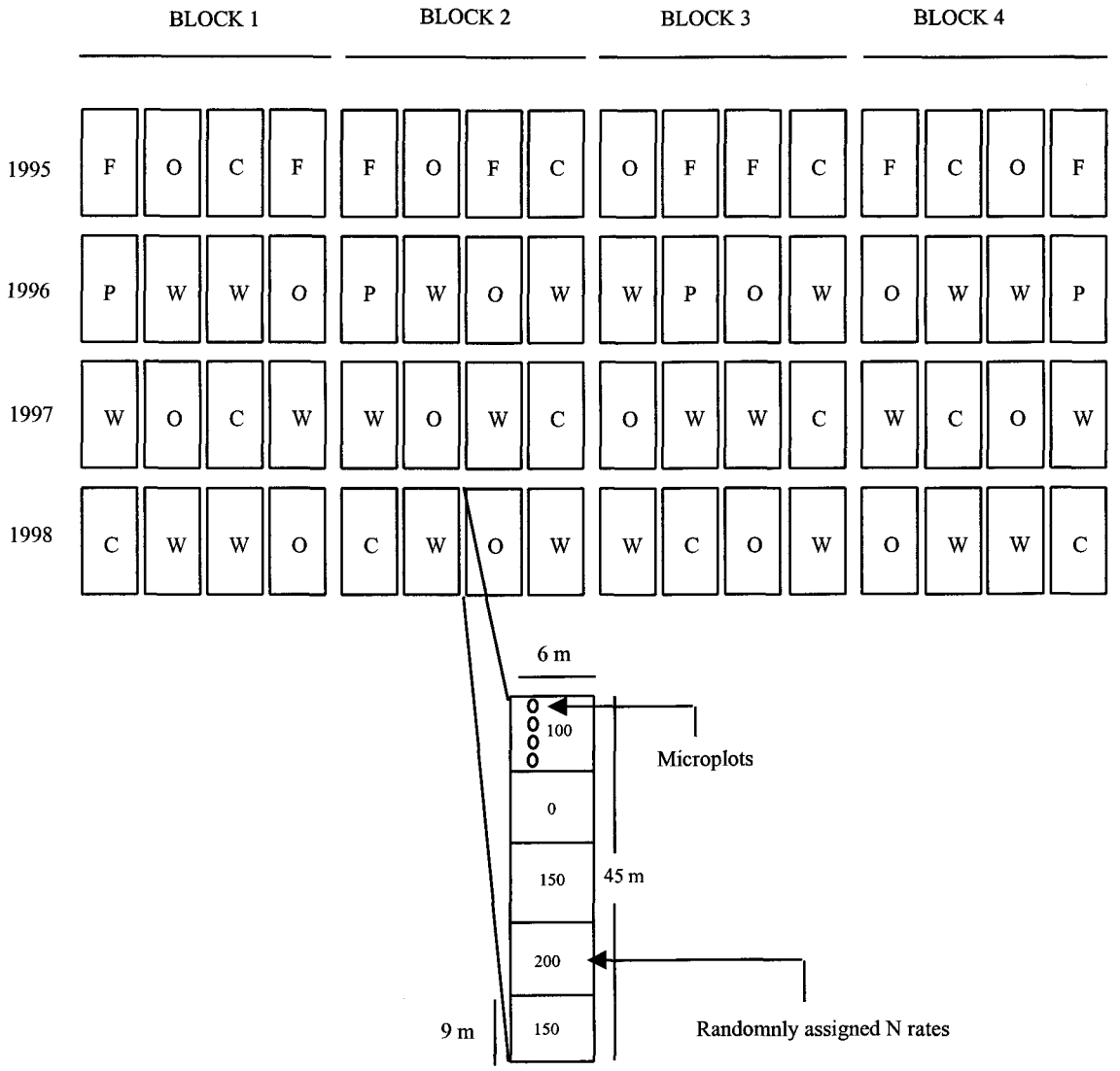

Figure 2-1. Cropping sequence, for the growing seasons of 1995-96, 1996-97, and 1997-98, layout of fertilizer treatments, and location of micro plots. F = fallow, C = clover, P = pea, O = oats, and W = wheat.

RESULTS AND DISCUSSION

Dry matter yield:

The influence of previous crop and nitrogen fertilizer on biomass accumulation in the micro plots in three growing seasons is illustrated by data in (Table 2-2). Wheat after legume accumulated more biomass than wheat following oat in all three growing seasons. Differences in biomass accumulation due to previous crop were significant at the time of fertilizer application (Feekes GS 4) in the 1996-97 and 1997-98 growing seasons. Though the trend was the same in 1995-96, differences due to previous crop were not significant at the time of fertilizer application. However, in all three growing seasons significant differences in biomass accumulation due to previous crop were observed in both fertilized and unfertilized plots at Feekes GS 8, the time when crop had accumulated about 1450 growing degree days (GDD). Wheat after legume produced significantly more biomass than wheat after oats in both fertilized and unfertilized plots.

The biomass yield response to nitrogen was substantial in both rotations in 1995-96 and 1996-97 growing seasons. Biomass yield increased significantly with application of 100 kg N ha^{-1} irrespective of rotation. Biomass increase from applied N ranged 4.8 Mg ha^{-1} to 4.6 Mg ha^{-1} for wheat after legume and wheat after oat, respectively in 1995-96. In 1996-97, the increase of biomass due to nitrogen fertilization was 5.6 Mg ha^{-1} and 4.8 Mg ha^{-1} for wheat after legume and wheat after oat, respectively. Although nitrogen fertilizer significantly increased biomass across rotations, the effect was more pronounced in 1996-97 where wheat followed legume. The interaction of previous crop with nitrogen

application was not statistically significant in 1996-95 and 1997-98 growing seasons. In contrast to the previous two seasons, nitrogen fertilizer application had less effect on biomass accumulation in 1997-98; the average increase in biomass due to nitrogen fertilizer was only 1.2 Mg ha^{-1}. As all micro plots received nitrogen fertilizer, the interaction effects of rotation with nitrogen application could not be evaluated in 1997-98.

The impact of previous crop on biomass accumulation measured in large plots later in the season (Feekes GS 11.4) was consistent with that of micro plots. In all three growing seasons, wheat after legume accumulated more biomass than wheat after oat in both fertilized and unfertilized plots (Table 2-3). Consistent with the micro plots, differences due to previous crop were significant for unfertiliized plots in 1996-97 and 1997-98. The unfertilized plots showed a greater impact of previous crop than the fertilized plots in all growing seasons. The impact of previous crop in fertilized plots was 7, 54 and 6 % as large as the impact in unfertilized plots in 1995-96, 1996-97 and 1997-98 respectively. In fertilized plots, previous crop differences were statistically significant only in the 1996-97 growing season.

Crop response to applied nitrogen was substantial and significant in both rotations in all three growing seasons. However, the response to N fertilizer was greater for wheat after oat than for wheat after legume in all seasons. The biomass yield increase due to N fertilizer in wheat following oat was 10, 14 and 13 Mg ha^{-1} in 1995-96, 1996-97 and 1997-98 respectively, whereas, in wheat following legume the response to applied N in all three seasons averaged 9 Mg ha^{-1}.

Biomass accumulation with time followed a sigmoidal pattern during all three growing seasons. Sigmoidal growth curves for three growing seasons are illustrated in (Figures 2-2 through 2-4). Estimated parameters of the growth curve, maximum growth rate, and times at which maximum growth rate and 90 % of total biomass accumulation were observed are shown in Table 2-4. During all growing seasons a very sharp increase in biomass accumulation was observed after the crop had accumulated 1250 GDD. However, fertilized wheat grew at a faster rate than the unfertilized wheat in both rotations in all three seasons. Across rotations, the rate of biomass accumulation in fertilized plots was about 3 times faster than in unfertilized plots. In all three growing seasons, 90 % of total biomass was accumulated between 1990 and 2700 GDD. Wheat after legume tended to accumulate 90 % biomass earlier than wheat after oat. Averaged over three seasons, 90 % of the total biomass accumulation occured 100 GDD earlier in wheat following legume as compared to wheat following oat. In 1995-96 (Fig 2-2), crop rotation had little affect the on rate of biomass accumulation in either fertilized or unfertilized plots. The maximum rate of biomass accumulation in unfertilized plots was 6×10^{-3} and 4×10^{-3} kg ha^{-1} day^{-1} in wheat following legume and wheat following oat, respectively, whereas in the fertilized plots the maximum rate of biomass accumulation was 23×10^{-3} and 22×10^{-3} kg ha^{-1} GDD^{-1} in wheat after legume and wheat after oat, respectively.

In the 1996-97 growing season (Fig 2-3), the effect of the previous crop was small in early season and tended to increase as the crop grew in both fertilized and unfertilized plots. The impact of previous crop was more pronounced in unfertilized plots than in

fertilized plots at the time of maturity (3000 GDD). In unfertilized plots, the maximum rate of biomass accumulation of wheat after legume was twice as high but occurred 136 GDD later than for wheat after oat. Similarly, the wheat after oat reached 90 % biomass accumulation 320 GDD earlier than wheat after legume. Conversely, the maximum rate of biomass accumulation in the fertilized plots, was only 1.3 times higher in wheat after legume as compared to wheat after oat. Both maximum growth rate and 90 % biomass accumulation was observed about 100 GDD earlier in wheat following legume than the wheat after oat.

Similar to 1995-96, the effect of previous crop in fertilized plots during 1997-98 was small in early season and almost diminished at maturity in the fertilized plots (Fig 2-4). The rates of maximum biomass accumulation in the fertilized plots was 14.3 and 13.6 x 10^{-3} kg ha^{-1} GDD^{-1} for wheat after legume and wheat after oat, respectively. Compared to the previous two seasons, the maximum biomass accumulation rates in fertilized plots were lower, and the biomass kept accumulating until the end of the season. In fertilized plots, 90 % biomass was accumulated about 500 GDD later than in the previous two seasons. The rotation effect in unfertilized plots was similar to that measured in 1996-97 but biomass yields were greater. The maximum biomass accumulation rate in unfertilized wheat following oat was more than twice the rate observed for unfertilized wheat after oat. Similar to the previous two seasons, both rotations in the unfertilized plots accumulated 90 % biomass between 1900 and 2450 GDD.

In all growing seasons, the effect of the previous crop on wheat biomass was more pronounced in micro plots than in the large plots (Table 2-2, 2-3). For example, wheat biomass after oat in fertilized micro plots was 81, 65 and 70 % of wheat biomass following legume in three growing seasons. In contrast, wheat biomass after oat in fertilized large plots was 99, 83 and 98 % of wheat biomass after legume. This indicates that as the crop grew the rotation effects on biomass almost disappeared. Also because the growth in micro plots was more homogeneous than that of large plots, the impact of previous crop on the biomass accumulation was estimated more precisely in micro plots as compared to that of large plots. The coefficient of variation (CV) across three seasons averaged 10 %, and 16 % in fertilized micro plots and large plots, respectively. In the second year of rotation, 1996-97, wheat after oat did not catch up, indicating a more N deficient environment in the second year of rotation than in the first year. In 1997-98 the rotation effects were almost similar to that of 1995-96 in the large fertilized plots. This is because in 1997-98 both rotations received 20 kg N ha^{-1} banded with seed at planting which might have alleviated early season N deficiency in the wheat-oat rotation. The spring N in 1997-98 did not show as big an impact on biomass yield as it had in the previous two seasons in the micro plots. On the other hand, in the large plots the effect of spring N was nearly the same as observed in the previous two seasons.

Table 2-2. Impact of previous crop on biomass yield of winter wheat in micro plots as influenced by time and nitrogen fertilizer application in three growing seasons.

Growing season	Time after fertilization	GDD†	N rate	Biomass following: Legume	Oat	Difference (SE_{diff})‡
	days		----------kg ha^{-1}----------			
1995-96						
	0	961	0	637A	519A	118 (154)
	50	1455	0	2781c	1567d	1214 (581)
	50	1455	100	7570a	6150b	1420 (676)
1996-97						
	0	999	0	980A	181B	799 (9.5)
	68	1614	0	4126c	1474d	2652 (425)
	68	1614	100	9744a	6288b	3456 (437)
1997-98						
	0	879	0	1466A	654B	812 (105)
	72	1509	0	6570A	3020B	3550 (460)
	72	1509	100	7147A	4978B	2169 (335)

†Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C).
‡$SE_{(df=3)}$ of the difference between two means at the same N rate and GDD. Within a growing season, values followed by the same capital letter are not significantly different at $\alpha = 0.05$. To compare N rates within previous crop at the same GDD, use $SE_{dAB(df=6)}$ = 228 for 1995-96 or $SE_{dAB(df=6)}$ = 396 for 1996-97. Use $SE_{d(df=3)}$= 635 for 1995-96 or $SE_{d(df=6)}$= 481 for 1996-97 to compare within or between columns at the same GDD. Within a growing season values followed by same lower case letter are not significantly different at $\alpha = 0.05$.

Table 2-3. Impact of previous crop on biomass yield of winter wheat in large plots as influenced by nitrogen fertilizer application in three growing seasons.

Growing season	Time after fertilization	GDD†	N rate	Biomass following: Legume	Oat	Difference (SE_{diff})‡
	days		----------kg ha^{-1}----------			
1995-96						
	130	2763	0	7140b	5660b	1980 (1903)
	130	2763	100	15980a	15836a	144 (1436)
1996-97						
	154	3020	0	9420c	2965d	6455 (673)
	154	3020	100	20815a	17245b	3470 (1709)
1997-98						
	163	3076	0	12533b	7049c	5484 (620)
	163	3076	100	20144a	19830a	314 (903)

†Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C).
‡$SE_{(df=3)}$ of the difference between two means at the same N rate. To compare N rates within previous crop at the same GDD, use $SE_{dAB(df=6)}$ = 1930 for 1995-96, $SE_{dAB(df=6)}$ = 1160 for 1996-97 or $SE_{dAB(df=6)}$ = 473 for 1997-98. Use $SE_{d(df=9)}$= 1715 for 1996, $SE_{d(df=8)}$= 1102, for 1997 or $SE_{d(df=6)}$= 441 for 1998 to compare within or between column at the same GDD. Within a growing season values followed by same lower case letter are not significantly different at $\alpha = 0.05$.

Table 2-4. Estimates of the parameters for sigmoidal model describing biomass accumulation of winter wheat, maximum rate of accumulation, and time of maxim rate and 90 % biomass accumulation as influenced by nitrogen fertilization and previous crop (legume or oat) in three growing seasons.

	1995-96				1996-97				1997-98			
	0 kg N ha^{-1}		100 kg N ha^{-1}		0 kg N ha^{-1}		100 kg N ha^{-1}		0 kg N ha^{-1}		100 kg N ha^{-1}	
	Legume	Oat	Legume	Oat	Legume	Oat	Legume	Oat	Legume	Oat	Legume	Oat
R^2	**0.80**	**0.90**	**0.92**	**0.98**	**0.97**	**0.97**	**0.98**	**0.96**	**0.97**	**0.95**	**0.97**	**0.98**
K	**7263**	**6158**	**15987**	**15850**	**9651**	**2970**	**20849**	**17330**	**12588**	**6939**	**20472**	**20903**
SE†	914	1569	1047	500	391	102	597	755	354	338	984	859
N_0‡	**28.37**	**43.67**	**2.17**	**2.35**	**75.99**	**1.86**	**10.07**	**7.77**	**6.56**	**22.24**	**134**	**105**
r	**3.49E-3**	**2.66E-3**	**6.00E-3**	**5.70E-3**	**2.82E-3**	**4.56E-3**	**4.60E-3**	**4.40E-3**	**5.00E-3**	**3.53E-3**	**2.80E-3**	**2.60E-3**
SE	2.63E-3	2.04E-3	3.3E-3	1.9E1-3	0.49E-3	0.69E-3	0.64E-3	1.2E-3	0.85E-3	0.76E-3	0.41E-3	0.26E-3
$r_{(max)}$¶	**6.3E-3**	**4.1E-3**	**23.9E-3**	**22.5E-3**	**6.8E-3**	**3.4E-3**	**23.9E-3**	**19.0E-3**	**15.7E-3**	**6.1E-3**	**14.3E-3**	**13.6E-3**
$GDD_{(max)}$§	**1611**	**1861**	**1511**	**1561**	**1750**	**1614**	**1699**	**1799**	**1569**	**1668**	**1819**	**2069**
$GDD_{(0.9)}$£	**2172**	**2446**	**1849**	**1931**	**2416**	**2096**	**2133**	**2241**	**1943**	**2297**	**2523**	**2711**

†SE for the parameters has 9, 13, and 17 df for 1995-96, 1996-97 and 1997-98 growing seasons respectively.
‡N_0 = K/(C+1), an estimate of initial or minimum biomass accumulation. ¶The rate of maximum biomass accumulation (kg/ha/GDD).
§Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C), $GDD_{(max)}$ is growing degree days when the maximum rate of biomass accumulation occurred.
£$GDD_{(0.9)}$ is growing degree days at which 90 % of the seasonal total biomass had accumulated.

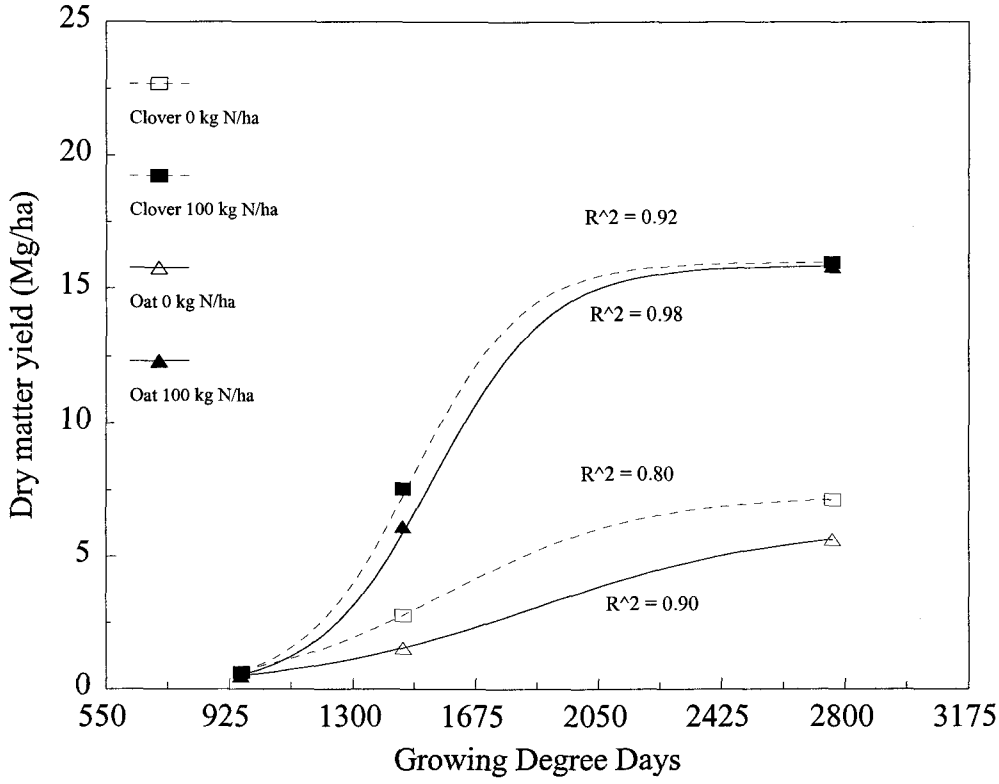

Figure 2-2. Biomass accumulation of wheat as influenced by previous crop and nitrogen fertilizer application during 1995-96 growing season.

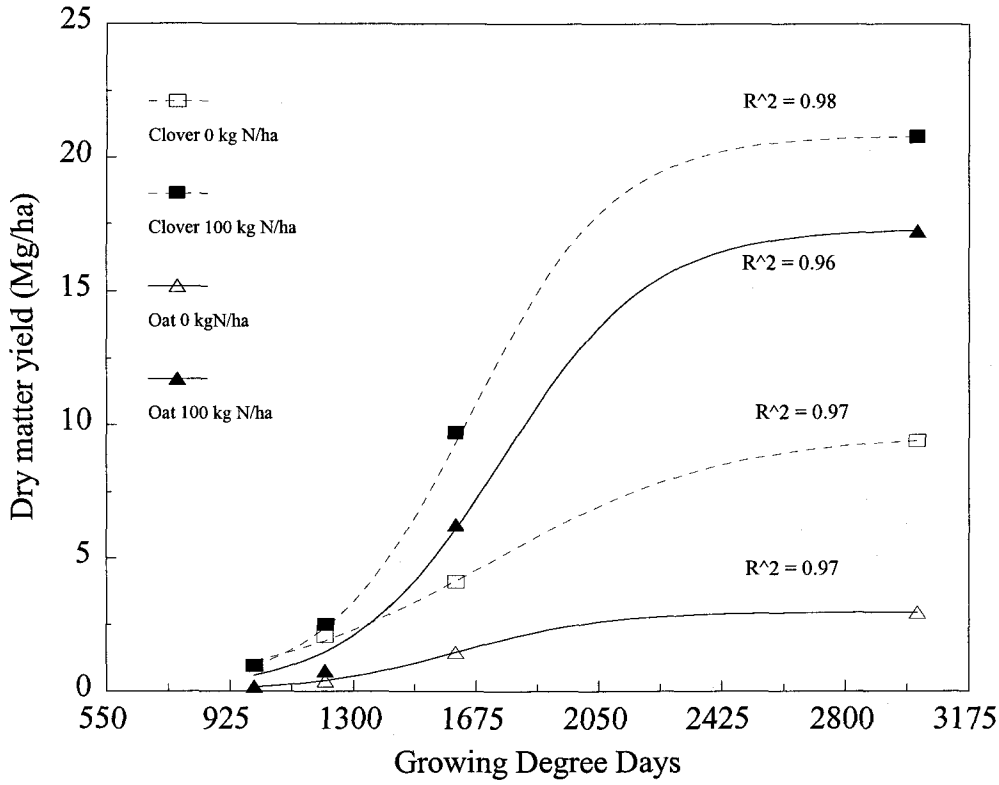

Figure 2-3. Biomass accumulation of wheat as influenced by previous crop and nitrogen fertilizer application during 1996-97 growing season.

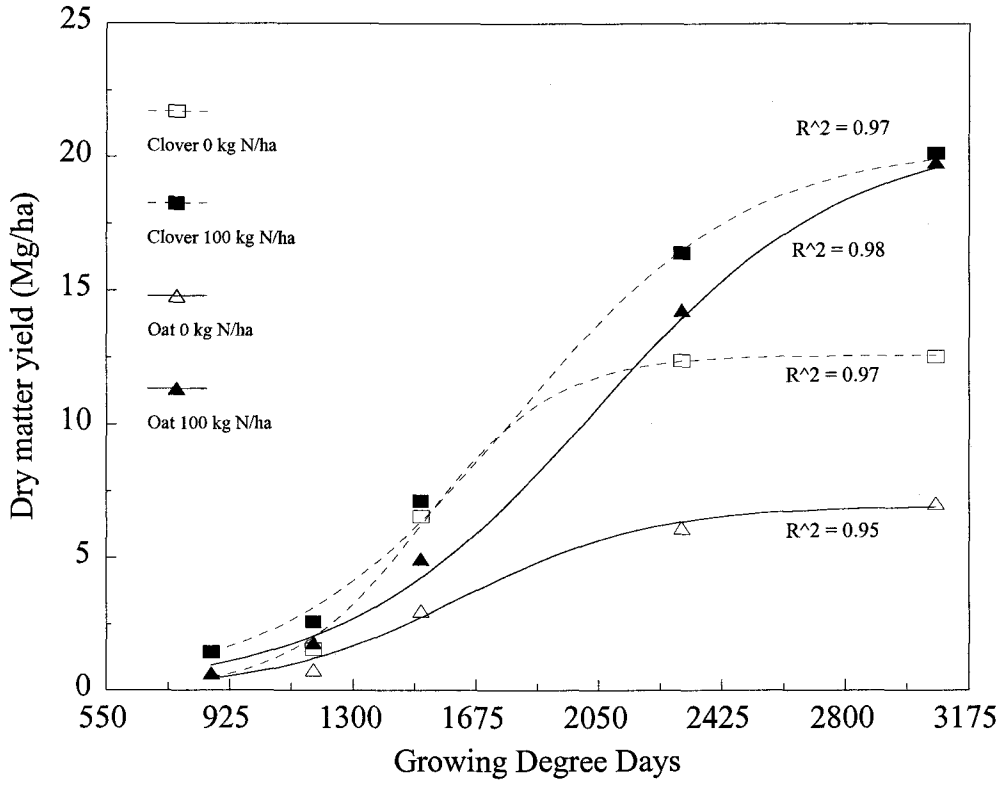

Figure 2-4. Biomass accumulation of wheat as influenced by previous crop and nitrogen fertilizer application during 1997-98 growing season.

Nitrogen uptake:

The influence of previous crop on nitrogen uptake by wheat in micro plots in the three growing seasons is illustrated in (Table 2-5). In all three seasons wheat following legume took up more nitrogen than wheat after oat. Nitrogen uptake was increased from 6 to 67 kg N ha^{-1} across the three growing seasons where wheat followed a legume. The impact of previous crop on N uptake was evident as early as Feekes GS 4, (before the crop had accumulated 1000 GDD) and ranged from 6 kg N ha^{-1} in 1995-96 to 34 kg N ha^{-1} in 1997-98. However, differences between the two rotations at Feekes GS 5 were only statistically significant in the 1996-97 and 1997-98 growing seasons. After the crop had accumulated 1450 GDD (Feekes GS 8) significant differences due to rotation were observed in both fertilized and unfertilized plots in all three seasons. At Feekes GS 8, unfertilized wheat showed a consistent difference between legume-wheat and oat-wheat rotations during all growing seasons. The difference due to previous crop in the unfertilized wheat after 1450 GDD was 21, 27, and 26 kg N ha^{-1} in 1995-96, 1996-97 and 1997-98, respectively.

Interaction between previous crop and nitrogen application significantly affected nitrogen uptake in two out of three growing seasons (1995-96 and 1996-97). Interaction was such that differences in nitrogen uptake due to previous crop were enhanced when nitrogen fertilizer was added. For example, in 1995-96 the difference in nitrogen uptake between wheat following legume and wheat following oat was 21 kg N ha^{-1} in unfertilized plots, and increased to 47 kg N ha^{-1} in the fertilized plots. A similar pattern was observed in 1996-97, but with a higher magnitude of difference. The added nitrogen

interaction (ANI) increased to 40 kg N ha^{-1} in the 1996-97 growing season. In the 1997-98 growing season the ANI could not be evaluated because of different sampling procedures. However, the impact of nitrogen application on N uptake was very small averaging about 20 kg N ha^{-1} across rotations. When compared to the previous two seasons, the N uptake in the fertilized plots was much lower in both rotations during 1997-98. Compared to the mean for 1995-96 and 1996-97, 1997-98 increase in N uptake due to fertilization were only 19 % and 35 % as large for wheat following oat and wheat following legume, respectively.

The rotation effect on nitrogen uptake measured later in the season (Feekes GS 11.4) in the large plots is shown in (Table 2-6). Consistent with the micro plots, the N uptake by wheat following legume was higher than N uptake by wheat after oat during all three growing season in both fertilized and unfertilized plots. The difference in nitrogen uptake due to rotation ranged from 14 to 42 kg ha^{-1}. Nitrogen uptake differences attributable to roatation were atatistically significant only in the 1996-97 and 1997-98 growing seasons. Unlike the micro plots, there was no significant interaction between rotation and nitrogen fertilizer application. Averaged across theree growing seasons, N fertilization increased N uptake by 76 kg N ha^{-1} where wheat followed a legume and 72 kg N ha^{-1} where wheat followed oats. In other words, added nitrogen interaction (ANI) was not observed when crop N uptake was assesed after 2500 GDD. The observed means, and the means estimated by least square method (LSM) in the unfertilized plots, were in close agreement and showed a similar impact of previous crop on N uptake by

wheat. Unlike the results measured in micro plots, N uptake by fertilized plants in both rotations was nearly the same in each of the three years growing seasons.

Nitrogen uptake as a function of growing degree days was fitted to a sigmoidal curve as illustrated in Figures 2-5 through 2-7. Estimated parameters of N uptake curves, rates of maximum N uptake, and time at which maximum uptake rate and 90 % of the total N uptake were observed are shown in Table 2-7. Nitrogen uptake was rapid after 1000 GDD and preceded biomass accumulation in the fertilized plots during 1995-96 and 1996-97 growing seasons. On average, N uptake by fertilized plots was 90 % of season total by 1550 GDD, whereas the corresponding biomass accumulation at these GDD averaged 40 % across rotations and growing seasons.

During 1995-96, by the time the crop accumulated 1570 growing degree days (Feekes GS 9) nitrogen uptake in fertilized plots of wheat after legume was almost over, and was 80 % of the season total for wheat following oats and unfertilized wheat following legume. The impact of rotation on the time and rate of maximum N uptake was substantial in both fertilized and unfertilized plots. The maximum uptake rate in fertilized plots ranged 31.8 x 10^{-2} and 19.0 x 10^{-2} kg N ha^{-1} GDD^{-1} and occurred at 1355 and 1573 GDD in legume-wheat and oat wheat rotations, respectively. When compared to fertilized plots the rate of maximum N uptake in unfertilized plots was about 5 to 8 times smaller and occurred about 250 to 700 GDD later in legume-wheat and oat-wheat respectively. However, in unfertilized plots the rate of maximum N uptake by wheat following legume was twice as high as that of wheat after oat.

During 1996-97, the rate of maximum N uptake in wheat following legume was twice that of wheat following oat in the fertilized plots. The rate of maximum N uptake was 23.7 x 10^{-2} and 12.1 x 10^{-2} kg N ha^{-1} GDD^{-1} in legume-wheat and oat-wheat, respectively. Likewise, 90 % of total N uptake was 470 GDD earlier in legume-wheat than in oat-wheat rotation. Consistent with 1995-96, N uptake in unfertilized plots was 5 to 9 times slower than that of fertilized plots. While the impact of previous crop on the rate of maximum N uptake in unfertilized plots was very small, 90 % N uptake by wheat after legume was reached 262 GDD later than by wheat following oat.

In contrast to previous two growing seasons, in 1997-98, though the total N uptake in 1997-98 was similar to that observed in the previous two seasons, N uptake did not precede biomass accumulation. The rate of maximum nitrogen uptake when compared to previous two years was much lower in 1997-98 in the fertilized plots of both rotations and was unaffected by rotation in both fertilized and unfertilized plots. The crop in both rotations of fertilized and unfertilized plots kept accumulating N till the end of the season.

The influence of previous crop on nitrogen uptake was comparable at Feekes GS 4 (850 to 1000 GDD) and Feekes GS 9 (1500 to 1600 GDD) in the 1996-97 and 1997-98 growing seasons (Table 2-5). This indicates that legume N was available throughout early growth, suggesting that N mineralization occurred very early in the season, possibly between late fall and early winter. This data is also supported by the biomass accumulation data which indicate that the difference in biomass due to rotation in two out of three years was evident before spring N fertilization (Table 2-2). These findings

contradict the hypothesis that the possible time for N mineralization in western Oregon is late winter or spring.

Unlike the precision in estimating biomass, the precision in estimating N uptake was almost the same in micro plots and large plots. The coefficient of variation (CV) averaged 16 and 17 % across seasons, rotations and N rates in micro plots and large plots, respectively. During all three seasons the impact of previous crop on nitrogen uptake was comparable in micro plots and large plots. Also, the impact of N fertilizer on the N uptake in the micro plots and large plots was in good agreement in 1995-96 and 1996-97. In contrast to that, micro plots and large plots gave different results in 1997-98 with respect to the impact of N fertilizer on crop growth and N uptake.

Table 2-5. Impact of previous crop on nitrogen uptake by winter wheat in micro plots as influenced by time and nitrogen fertilizer application in three growing seasons.

Growing season	Time after fertilization	GDD†	N rate	N-uptake following: Legume	Oat	Difference (SE_{diff})‡
	day		----------kg N ha^{-1}----------			
1995-96						
	0	961	0	18A	12A	6 (4.5)
	50	1455	0	44c	23d	21 (8.3)
	50	1455	100	134a	87b	47 (6.6)
1996-97						
	0	999	0	32A	6B	26 (0.5)
	68	1614	0	45c	18d	27 (5.5)
	68	1614	100	140a	73b	67 (8.7)
1997-98						
	0	879	0	56A	22B	34 (4.7)
	72	1509	0	56A	30B	26 (1.8)
	72	1509	100	74A	51B	23 (6.4)

†Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C).
‡$SE_{(df=6)}$ of the difference between two means at the same N rate and GDD. Within a growing season at the same rate and GDD, values followed by same capital letter are not significantly different at $\alpha = 0.05$. To compare N rates within previous crop at the same GDD, use $SE_{dAB(df=6)} = 6.7$ for 1995-96 or $SE_{dAB(df=6)} = 4.97$ for 1996-97. Use $SE_{d(df=6)} = 8.79$ for 1995-96 or $SE_{d(df=5)} = 7.84$ for 1996-97 to compare within or between columns at the same GDD. Values followed by same lower case letter are not significantly different at $\alpha = 0.05$.

Table 2-6. Impact of previous crop on nitrogen uptake by winter wheat in large plots as influenced by nitrogen fertilizer application in three growing seasons.

Growing season	Time after fertilization	GDD†	N rate	N uptake following: Legume	Oat	Difference (SE_{diff})‡
			----------kg N ha^{-1}----------			
1995-96						
	LSM§	2763	0	67A	42A	25 (14.5)
	130	2763	0	59b	45b	14 (11.1)
	130	2763	100	133a	109a	23 (18.5)
1996-97						
	LSM	3020	0	56A	19B	37 (9.5)
	154	3020	0	58c	20d	38 (3.5)
	154	3020	100	144a	102b	42 (10.7)
1997-98						
	LSM	3076	0	62A	36B	27 (8.4)
	154	3076	0	67c	39d	28 (4.1)
	154	3076	100	136a	110b	26 (13.9)

†Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C). ‡$SE_{(df=3)}$ of the difference between two means at the same N rate. §Least square method (the estimates of intercept of regression line). Within a growing season, values followed by the same capital letter are not significantly different at $\alpha = 0.05$. To compare N rates within previous crop at the same day, use $SE_{dAB(df=6)} = 18.79$ for 1995-96, $SE_{dAB(df=6)} = 4.82$ for 1996-97 or $SE_{dAB(df=6)} = 8.3$ for 1997-98. Use $SE_{d(df=9)} = 15.28$ for 1995-96, $SE_{d(df=6)} = 7.97$ for 1999-97 or $SE_{d(df=6)} = 9.75$ for 1997-98, to compare within or between columns at same GDD. Values followed by the same lower case letter are not significantly different at $\alpha = 0.05$.

Table 2-7. Estimates of the parameters for sigmoidal model describing nitrogen uptake of winter wheat, maximum rate of N uptake, and time of maximum rate and 90 % N uptake as influenced by nitrogen fertilization and previous crop (legume or oat) in three growing seasons.

	1995-96				1996-97				1997-98			
	0 kg N ha^{-1}		100 kg N ha^{-1}		0 kg N ha^{-1}		100 kg N ha^{-1}		0 kg N ha^{-1}		100 kg N ha^{-1}	
	Legume	Oat	Legume	Oat	Legume	Oat	Legume	Oat	Legume	Oat	Legume	Oat
R^2	**0.67**	**0.80**	**0.89**	**0.95**	**0.57**	**0.85**	**0.88**	**0.96**	**0.34**	**0.66**	**0.81**	**0.84**
K	**59.54**	**48.26**	**136.78**	**109.16**	**57.23**	**20.46**	**142.17**	**100.49**	**78.91**	**41.25**	**152.01**	**117.20**
SE†	7.17	9.35	11.09	5.08	4.68	1.28	4.54	4.70	15.14	4.88	13.33	9.88
N_0‡	**0.57**	**2.13**	**0.003**	**0.02**	**9.58**	**0.07**	**0.12**	**0.13**	**19.77**	**7.30**	**11.08**	**3.27**
r	**3.93E-3**	**2.04E-3**	**9.400E-3**	**7.01E-3**	**2.09E-3**	**4.76E-3**	**6.12E-3**	**4.82E-3**	**1.46E-3**	**1.57E-3**	**1.96E-3**	**2.42E-3**
SE	1.87E-3	1.31E-3	5.0E-3	1.2E-3	1.3E-3	1.33E-3	1.8E-3	0.74E-7	1.44E-3	0.80E-3	0.63E-3	0.72E-3
$r_{(max)}$¶	**5.8E-2**	**2.5E-2**	**31.9E-2**	**190.0E-2**	**2.9E-2**	**2.43E-2**	**23.7E-2**	**12.1E-2**	**2.8E-2**	**1.62E-2**	**7.4E-2**	**7.0E-2**
$GDD_{(max)}$§	**1211**	**1511**	**1161**	**1261**	**1049**	**1199**	**1399**	**1149**	**919**	**1020**	**1319**	**1469**
$GDD_{(0.9)}$£	**1699**	**2334**	**1355**	**1573**	**1909**	**1647**	**1408**	**1881**	**1552**	**2115**	**2022**	**2172**

†SE for the perameters has 9, 13, and 17 df for 1995-96, 1996-97 and 1997-98 growing seasons respectively.
‡N_0 = K/(C+1), an estimate of initial or minimum nitrogen uptake. ¶The rate of maximun N uptake (kg/ha/GDD). §Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C), $GDD_{(max)}$ is growing degree days of maximum rate of N uptake. £$GDD_{(0.9)}$ is growing degree days at which 90 % of the seasonal total nitrogen uptake had occured.

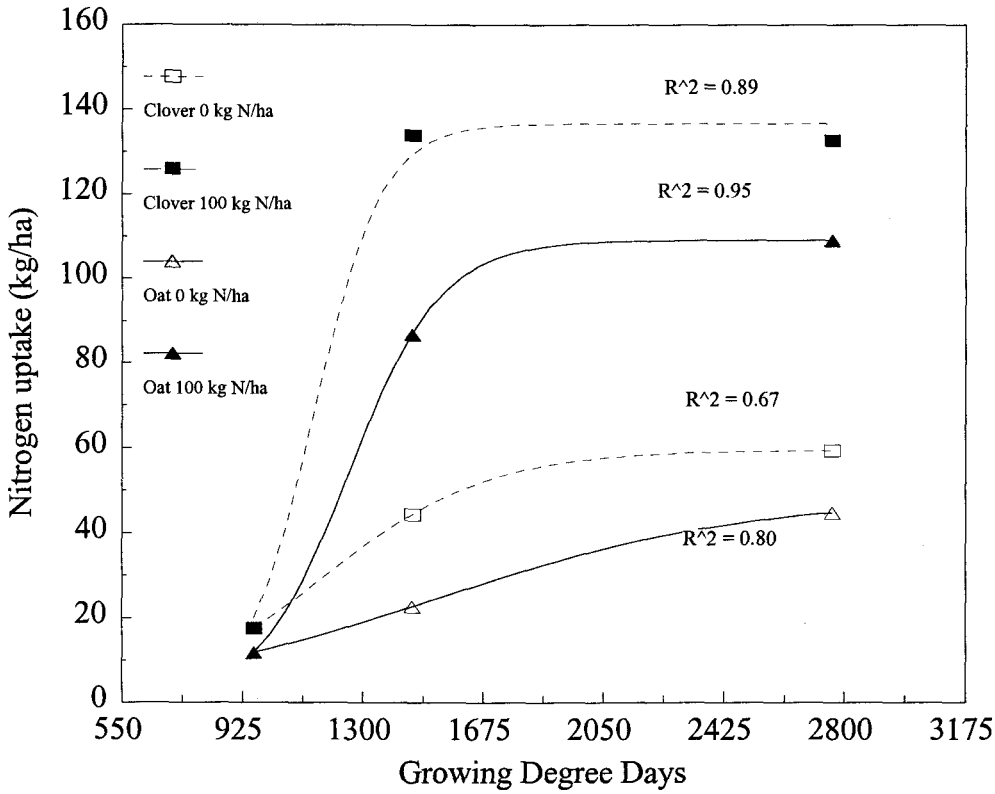

Figure 2-5. Nitrogen uptake of wheat as influenced by previous crop and nitrogen fertilizer application during 1995-96 growing season.

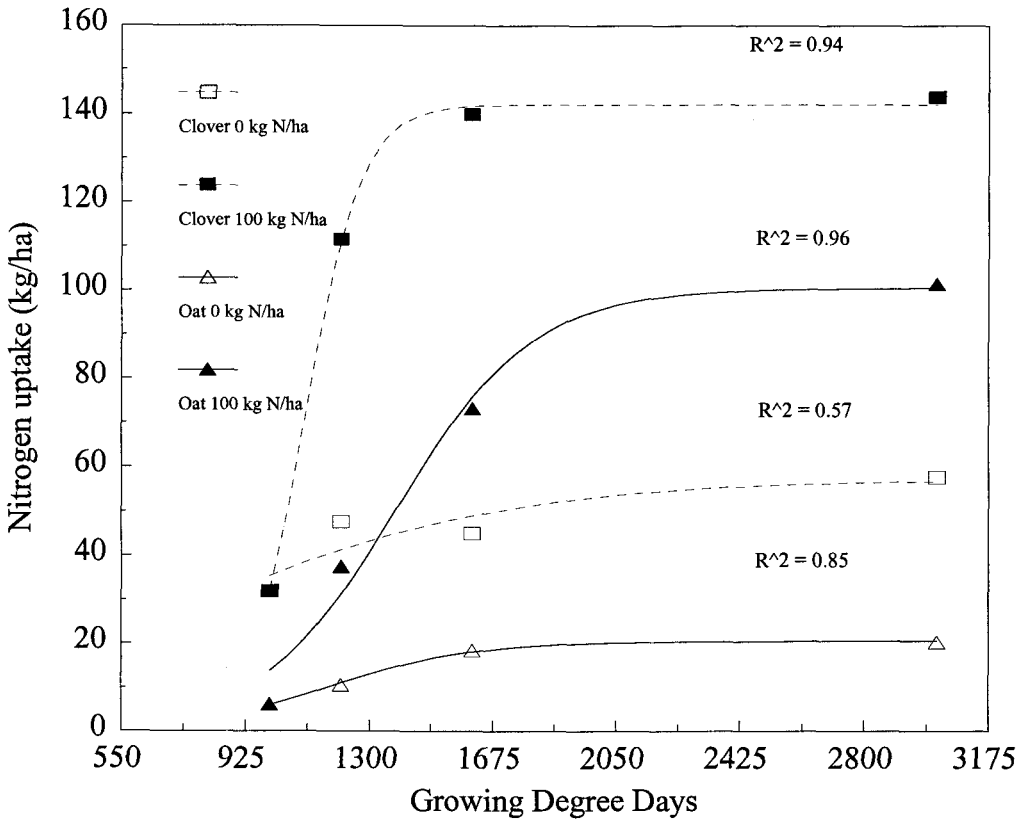

Figure 2-6. Nitrogen uptake of wheat as influenced by previous crop and nitrogen fertilizer application during 1996-97 growing season.

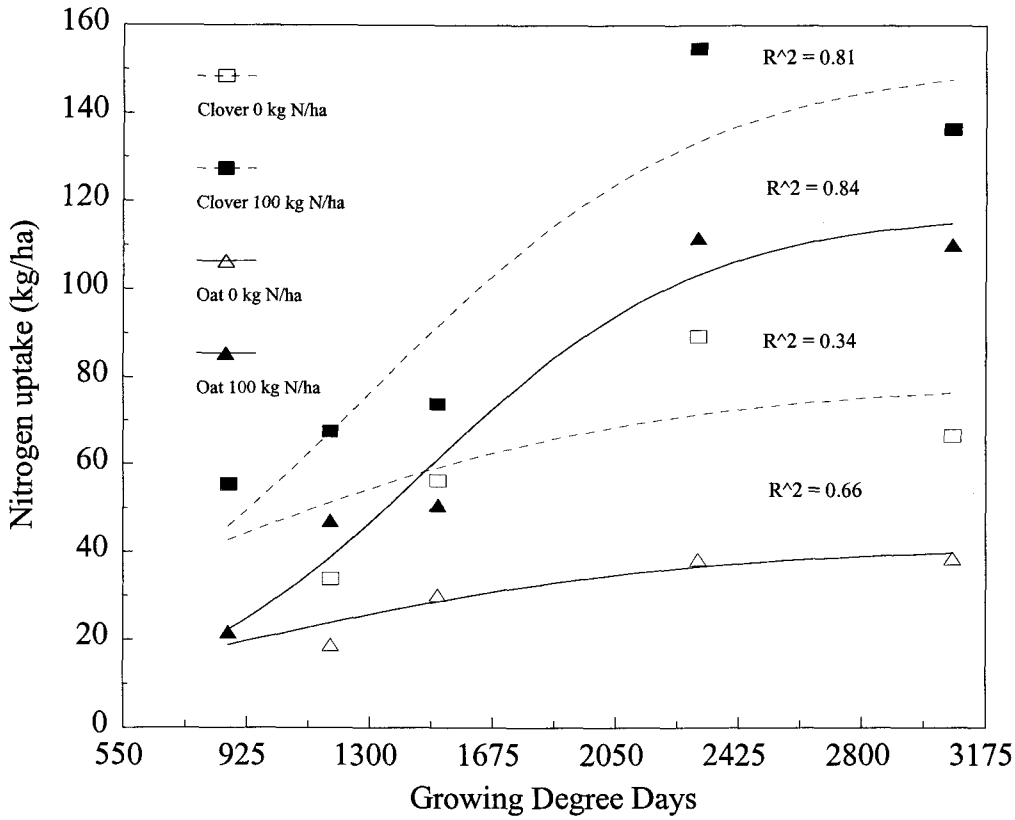

Figure 2-7. Nitrogen uptake of wheat as influenced by previous crop and nitrogen fertilizer application during 1997-98 growing season.

Nitrogen fertilizer use efficiency:

The impact of previous crop on N use efficiency estimated by isotopic and by difference method in micro plots is shown in Table 2-8. Nitrogen fertilizer use efficiency in the 1995-96 and 1996-97 growing seasons was significantly influenced by the previous crop. Wheat after legume showed a 21 to 39 % higher N use efficiency than wheat after oats during both growing seasons. In contrast, the N use efficiency was extremely low in and was unaffected by the previous crop in the 1997-98 growing season. In all years the nitrogen use efficiency estimated by isotopic dilution was consistent with that estimated by difference method.

From the data of biomass accumulation and nitrogen uptake which indicated that the wheat following oat was N deficient, it seemed that the wheat after oat would utilize applied N more efficiently, but the recovery data indicated otherwise. This suggests that either immobilization of applied nitrogen was greater in the wheat following oat system or the wheat plants following oat had been under nitrogen stress so long that the plant's ability to utilize fertilizer nitrogen was impaired.

Contrary to the literature which reported that the by difference method estimated higher nitrogen use efficiency than the isotopic method, our results showed that both isotopic and by difference methods were comparable. As was obvious from nitrogen uptake data that within 30 days of nitrogen fertilizer application the crop had taken up almost all of its nitrogen, the similarity in the two methods may be a result of this rapid N uptake, which did not allow fertilizer N to be exposed long enough for a substantial microbial N transformation. The data also suggest that in the spring, mineralization and

immobilization turnover (MIT) was not so rapid that it could resulted into a measurable ^{15}N dilution through the pool substitution in a 30 day period. In other words, it indicates that the mineralization of legume N had slowed by the time the crop received fertilizer nitrogen in the spring.

Nitrogen use efficiency estimated at the time of maturity (Feekes GS 11.4) using the by difference method was not significantly influenced by the previous crop (Table 2-9). Crop N uptake was linear with the amount of fertilizer added in the three growing seasons. Thus provided the opportunity to estimate N use efficiency as a slope of the regression line by least square method (LSM). Similar to the simple by difference method, the LSM showed no significant effects of previous crop on N use efficiency. However, the LSM showed smaller magnitude of rotation difference and, as evident by smaller standard error, was more precise than simply by difference method.

The impact of previous crop on N use efficiency was smaller at maturity than that observed at Feekes GS 8-9 in micro plots during 1995-96 and 1996-97 growing seasons. The difference due to previous crop on the N use efficiency estimated by difference method in the two seasons averaged 32 % and 7 % in micro and large plots respectively. In 1995-96 and 1996-97 growing seasons, the N use efficiency estimated by difference in large plots of wheat following legume was smaller than that estimated earlier in the season in micro plots by the same method. On the other hand, the N use efficiency in wheat following oat in 1995-96 remained the same as estimated early in the season in micro plots. But in 1996-97, the N use efficiency in wheat following oat was 26 % higher in large plots than estimated by difference method in micro plots. In general, the N use

efficiencies estimated in large plots were comparable over three years. In contrast, comparable results in the micro plots were observed only in the 1995-96 and 1996-97 growing seasons.

The 1997-98 season was an unusual season as heavy rains occurred just after fertilizer application and continued intermittently for the next thirty days. Within a month of fertilizer application, 15-cm rain fell, out of which 12-cm fell within fifteen days of fertilizer application (Fig 2-10). Whereas, in the previous two seasons the rain fall after fertilizer application was not as frequent as in 1997-98 (Fig 2-8 and 2-9). Since the micro plots represented a confined system, these rains had a greater effect on micro plots than on large plots. Also, micro plots received NH_4NO_3 which is more readily denitrified or leached than urea applied to the large plots. The heavy rains caused water ponds in the micro plots thereby causing losses of applied N, due to denitrification and/or NO_3 leaching. This is why the N uptake and NUE data collected from micro plots in 1997-98 was so radically different from that of the previous two years. On the other hand, the large plots were less affected by rains and data from large plots was consistent across the three growing seasons.

Fertilizer use efficiency estimates from large plots were higher than often reported in the literature in all three seasons. Fertilizer use efficiency averaging 73 % are probably a consequence of conditions where N was extremely limiting, even where a legume had been grown the year before. In growing seasons where data was reliable, NUE estimates from micro plots ranged from 53 to 94 % with an average of 75 %. Very high NUE estimates particularly in the micro plots is due to the short interval between fertilizer

application and the estimation of nitrogen use efficiency. The NUE reported in the literature is often estimated at the end of the season. When ^{15}N stays in the soil for a large duration, it is subject to dilution through mineralization of organic N, which results in a lower recovery of labeled N. Rather than waiting until the end of the growing season, NUE was estimated in micro plots only 50 to 70 days after fertilizer application (Feekes GS 8-9). As discussed previuosly, the mineralization of legume N had already slowed down by the time of fertilizer application and thus pool substitution through MIT was very small. Moreover, the uptake of applied N was so fast that almost all of the fertilizer N was taken up within 30 days. As a consequence, the recovery estimated by both isotopic and by difference method was higher than often reported.

Table 2-8. Impact of previous crop on nitrogen use efficiency (NUE) of winter wheat in micro plots estimated using labeled N and by difference methods in three growing seasons, at Feekes GS 8 to 9.

Growing season	Method	GDD†	NUE following: Legume	Oat	Difference (SE_{diff})‡
			--------- % ---------		
1995-96					
	50-day by ^{15}N	1455	94a	73b	21 (3.8)
	50-day by difference	1455	89a	64b	25 (2.5)
1996-97					
	68-day by ^{15}N	1614	81a	53b	28 (5.1)
	68-day by difference	1614	94a	55b	39 (4.2)
1997-98					
	72-day ^{15}N	1509	24a	22a	2 (1.1)
	72-day by difference	1509	31a	21a	10 (6.9)

†Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C).
‡$SE_{(df=3)}$ of the difference between two means within the same method. To compare methods within previous crop, use $SE_{d(df=14)}$ = 8.1 for 1995-96, $SE_{d(df=14)}$ = 10.1 for 1996-97 or $SE_{d(df=14)}$ = 4.7 for 1997-98. Within a growing season values followed by the same letter are not significantly different at $\alpha = 0.05$.

Table 2-9. Impact of previous crop on nitrogen use efficiency (NUE) of winter wheat in large plots estimated using Least Square and by difference methods in three growing seasons, at Feekes GS 11.4.

Growing season	Method	GDD†	NUE following: Legume	Oat	Difference (SE_{diff})‡
			--------- % ---------		
1995-96					
	130-day by LSM§	2763	65A	59A	6 (11.8)
	130-day by difference	2763	73a	64a	9 (26.6)
1996-97					
	130-day by LSM	3020	82A	79A	3 (7.7)
	130-day by difference	3020	86a	81a	5 (9.5)
1997-98					
	163-day by LSM	3076	74A	75A	-1(6.8)
	163-day by difference	3076	70a	71a	-1(13.4)

†Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C). ‡$SE_{(df=3)}$ of the difference between two means within the same method. §Least square method (the estimates of the slope of regression line). Within a growing season values followed by the same letter are not significantly different at $\alpha = 0.05$.

Soil nitrogen supply:

Soil nitrogen supply estimated 50 to 72 days after fertilizer application (Feekes GS 8-9) was significantly affected by previous crop over the three growing seasons (Table 2-10). The soil N supply was higher in wheat following legume as compared to wheat following oat in both fertilized and unfertilized plots. The nitrogen supplied by soil to unfertilized wheat averaged over years was 44 kg N ha^{-1} in wheat following legume and 24 kg N ha^{-1} in wheat following oat.

In 1996-97, the soil N supply was significantly affected by the interaction between rotation and N fertilizer. The soil N supply was higher in fertilized plots of wheat following legume (59 kg N ha^{-1}) as compared to that of unfertilized plots (45 kg N ha^{-1}). In contrast, nitrogen fertilizer did not influence the soil supply N in wheat following oat. These differences could be attributed to N cycling process from legume residue, which was stimulated by application of fertilizer nitrogen. In other words, the application of nitrogen had a catalytic effect on the mineralization immobilization turnover (MIT) in the plots which had readily mineralizable legume N. In 1997-98, soil N supply in wheat following oat appeared to increase an average of 10 kg N ha^{-1} in comparison to the previous two years. This increase may reflect the N added at planting only in 1997-98.

Over the three growing seasons, the soil N supply in unfertilized plots of wheat following legume remained almost constant, whereas in fertilized plots it showed an increase of 19 kg in 1997 and 10 kg in 1998 as compared to 1996. In wheat following oat on the other hand, soil N supply remained almost the same in both fertilized and

unfertilized plots, except in 1996. These trends might be a consequence of added nitrogen interaction (ANI), which was more pronounced after two years of legume-wheat rotation. This indicates that a positive added nitrogen interaction might be expected only where the soil already has enough readily available organically bound nitrogen, and the added fertilizer may not have any effects on soil N supply in nitrogen deficient systems such as wheat following oat for three consecutive seasons.

Table 2-10. Impact of previous crop on nitrogen supplied by soil to winter wheat in micro plots as influenced by nitrogen fertilizer application in three growing seasons.

Growing season	Time after fertilization	GDD†	N rate	Soil supplied N following: Legume	Oat	Difference (SE_{diff})‡
			----------kg N ha^{-1}----------			
1995-96						
	50	1455	0	44a	23bc	21 (8.3)
	50	1455	100	40ab	13c	27 (6.0)
1996-97						
	68	1614	0	45b	18c	27 (5.5)
	68	1614	100	59a	20c	38 (5.6)
1997-98						
	72	1509	0	56a	30b	26 (1.8)
	72	1509	100	50a	28b	22 (6.3)

†Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 °C).
‡$SE_{(df=3)}$ of the difference between two means at the same N rate. To compare N rates within previous crop at the same GDD, use $SE_{dAB(df=6)}$ = 7.48 for 1995-96, $SE_{dAB(df=6)}$ = 2.69 for 1996-97 or $SE_{dAB(df=6)}$ = 4.76 for 1997-98. Use $SE_{d(df=7)}$ = 8.54 for 1995-96, $SE_{d(df=4)}$ = 5.71 for 1996-97 or $SE_{d(df=7)}$ = 4.59 for 1997-98, to compare within or between columns at the same GDD. Within a growing season values followed by the same letter are not significantly different at $\alpha = 0.05$.

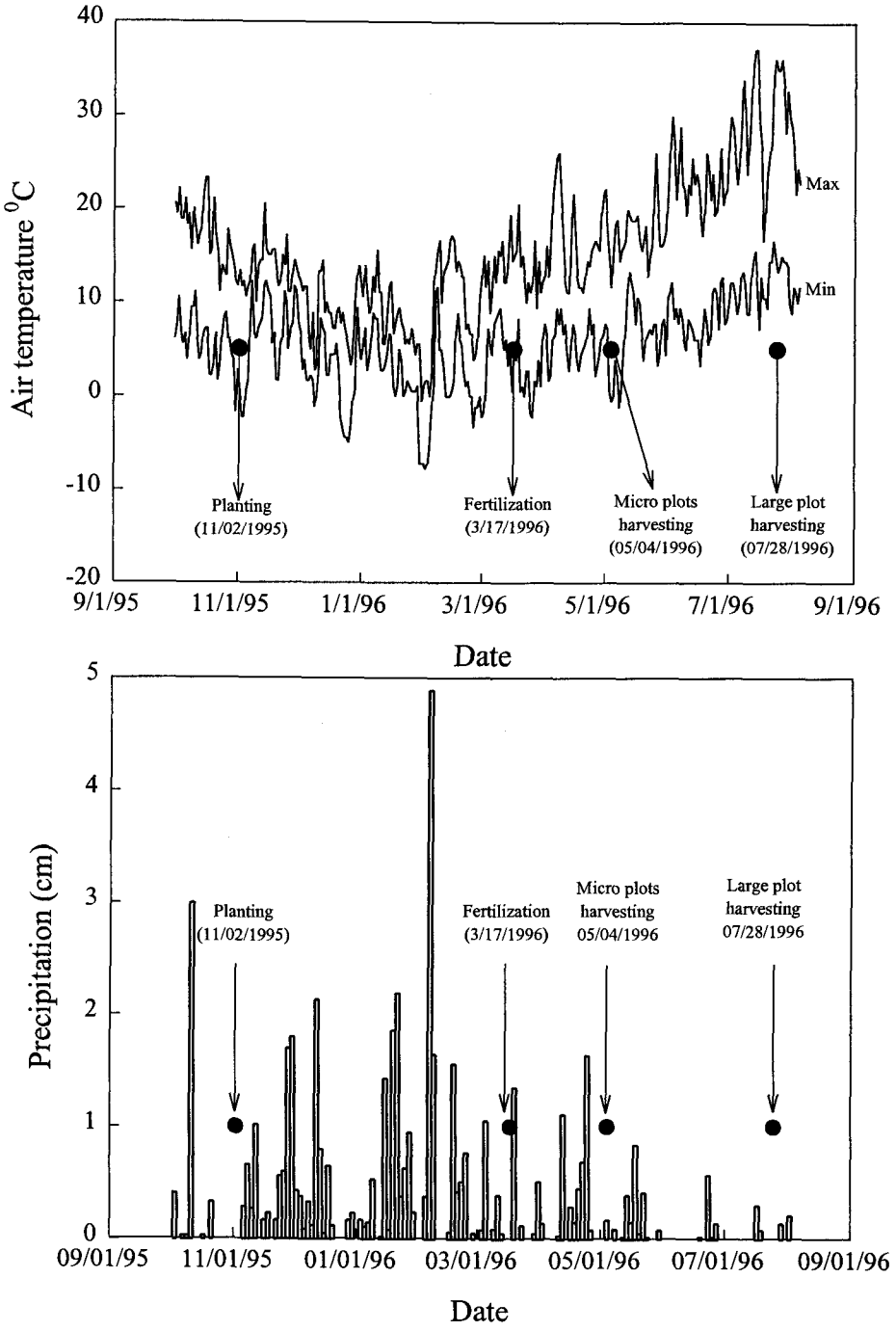

Figure 2-8. Minimum and maximum air temperature and precipitation during 1995-96 growing season. Solid circles indicate time of planting, fertilization and harvesting.

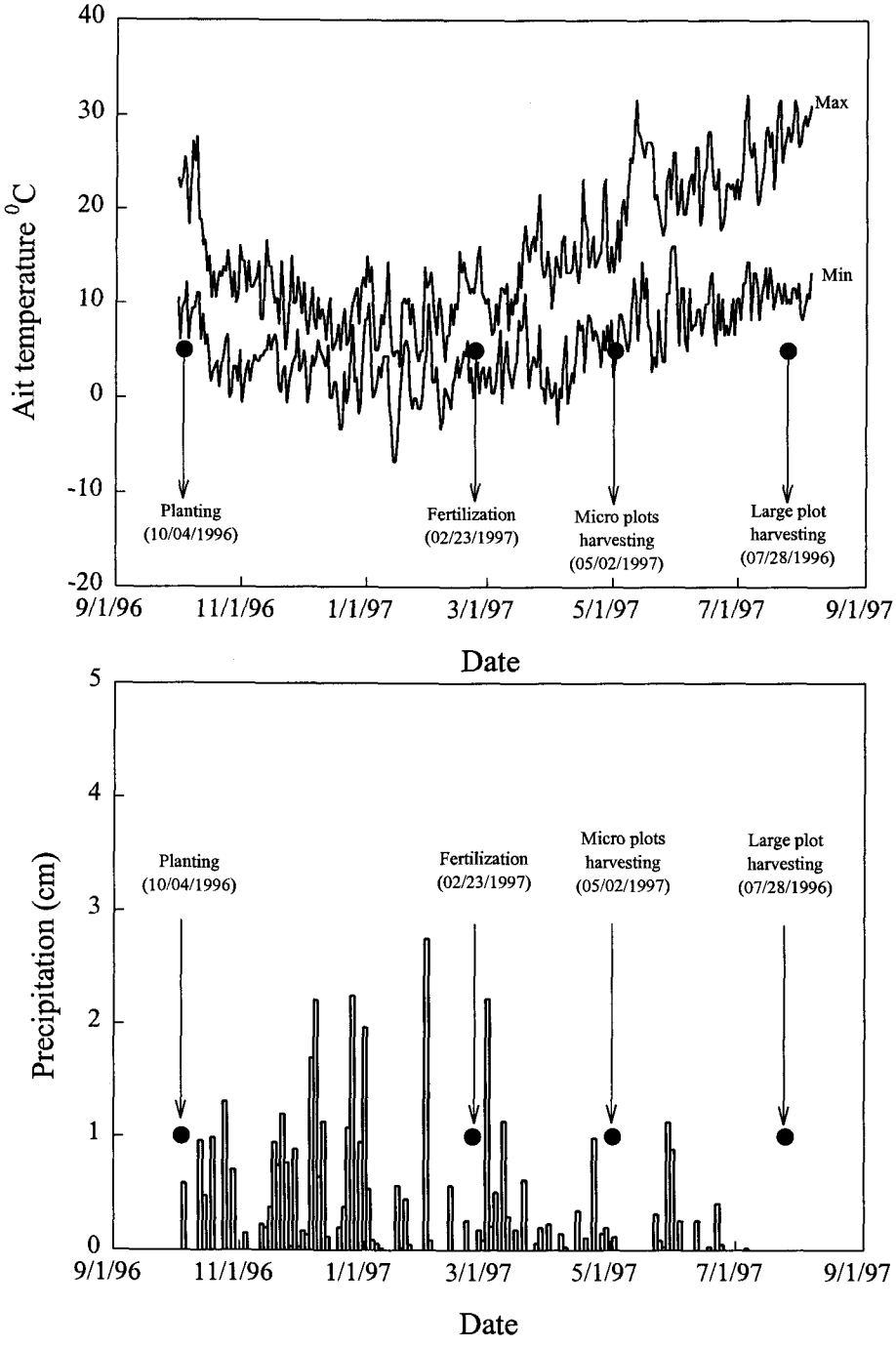

Figure 2-9. Minimum and maximum air temperature and precipitation during 1996-97 growing season. Solid circles indicate time of planting, fertilization and harvesting.

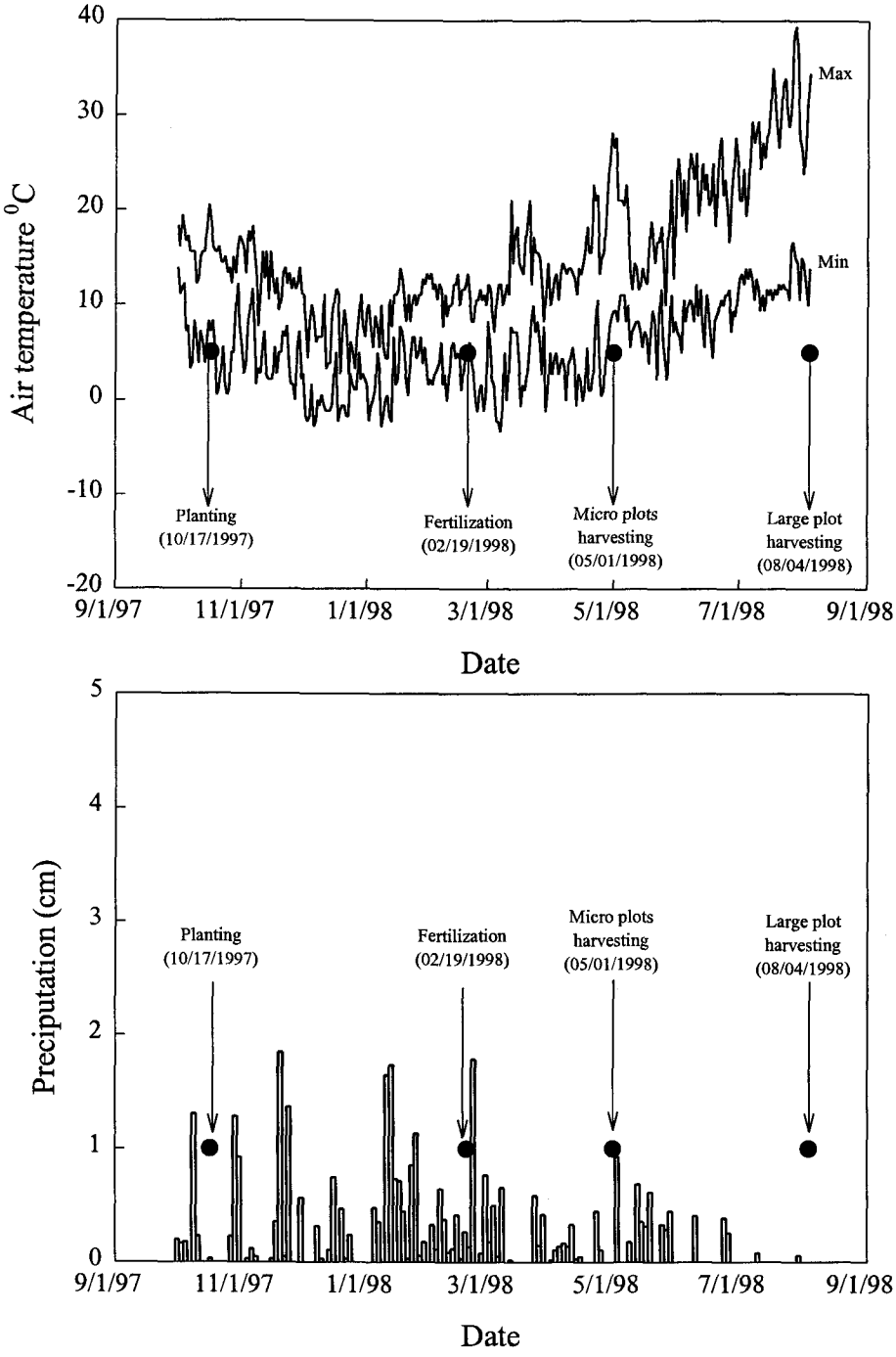

Figure 2-10. Minimum and maximum air temperature and precipitation during 1997-98 growing season. Solid circles indicate time of planting, fertilization and harvesting.

CONCLUSIONS

Over three cropping cycles, wheat showed increased growth, N uptake, and N use efficiency when grown after by legume as compared to after oat. The higher N use efficiency of wheat following legume may be a result of immobilization of applied N in wheat-oat plots or the physiological incapability of wheat following oat to utilize applied N due to prolonged N stress. Both isotopic and by differences method showed similar results in micro plots. This indicates that the N pool substitution was minimal in the 50-70 days duration, an evidence that a major portion of legume N had been mineralized sometime earlier in the season. The best time to evaluate the impact of previous crop on N use efficiency is 50 to 70 days after spring N fertilizer application. If the N use efficiency is evaluated within this time frame the isotopic method, which is more expensive and labor intensive, has little advantage over the by difference method which is relatively cheap and requires less labor. Nitrogen use efficiency estimated at the end of the season showed that the least square method (LSM) was in good agreement with the simple by difference method, but was more precise, and is a better choice if multiple N rates are used.

The effect of legume was evident as early as Feekes GS 4 in all three seasons, indicating that N from the legume residue mineralized and became available to the wheat in the fall or winter. Nitrogen derived from legume was estimated by dividing the difference of soil supplied N between wheat after legume and wheat after oat with the average NUE of the two rotation estimated by LSM. Contribution of legume N estimated

by the ratio of net soil N supply from legume to the N use efficiency was 44, 47 and 29 kg N ha^{-1} in the three seasons, respectively. Similar estimates were obtained from grain yield data taken from a contemporary study on the same experiment (Baloch, 1998). The grain yield followed a quadratic relationship with the amount of fertilizer added. Setting the intercept of wheat following legume equal to the regression equation of wheat following oat, and solving for the amount of fertilizer, gave an estimate of contribution of legume N (i.e., fertilizer replacement value). The fertilizer replacement values were 48, 58, and 36 kg N ha^{-1} in the three seasons, respectively. These estimates show that when wheat is planted after a legume, fertilizer rates can be reduced by 44 kg N ha^{-1} without any yield losses. These findings are compatible with the N fertilizer recommendations made in the fertilizer guide (FG 9) (Hart et al., 1992) for winter wheat following a legume in western Oregon. The stand of wheat after oat at early growth stages in the first two growing seasons was very poor due to N deficiency. Fall N application in 1997-98 alleviated N deficiency, implying that if the previous crop is oat, fall N application to winter wheat is necessary to avoid yield losses.

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CHAPTER 3

ESTIMATION OF NITROGEN TRANSFORMATION RATES USING ^{15}N LABELED MICROPLOTS

INTRODUCTION

Winter wheat is commonly rotated with a variety of crops in the Willamette Valley of western Oregon to diversify the cropping systems and to break weed and disease cycles. Among the other crops, legumes are also included in rotation schemes with winter wheat. The potential of annual legumes to conserve soil and water resource as an alternative to summer fallow and as a green manure for supplying plant nutrients has been well documented (Hargrove, 1988; Power, 1987). A legume crop grown prior to winter wheat may give several benefits including off-season soil cover, stimulated soil biological activity (Collins et al., 1992), improved soil fertility and plant nutrition (Breland, 1994), and depriving soil-borne wheat pathogens of a host (Kollmorgen et al., 1983). However, most interest has been attached to the ability of legume to furnish a subsequent crop with readily available N (Groffman et al., 1987). Therefore, generally the overall effects of the legume or other crops on the subsequent crop in terms of crop yield and nitrogen uptake are more emphasized. A considerable amount of research on the factors and conditions determining the mineralization in the soil has been conducted. Such research has been mainly directed to the developments of methods for predicting soil N supply to a crop on a year or half year basis in order to make fertilizer

recommendations (Mary and Recous, 1994). The new environmental constraints placed on agricultural production makes necessary to fully utilize the benefits of previous crops and minimize fertilizer N losses and disadvantages of excessive N fertilizer. Therefore, a better understanding of the magnitude and mechanisms of previous crop effects on wheat yield is needed. Nitrogen losses may also occur if mineralization does not coincide with plant uptake. To predict potential effects of N losses and the availability of N to the subsequent crop, knowledge about mineralization kinetics of the plant material added to the soil is essential which to allow growers to maximize the potential benefits within a cropping sequence.

The decomposition of organic matter in soil, and the accompanying mineralization and immobilization of inorganic N, are key processes in soil-plant N cycle (Watkins and Barraclough, 1995). Mineralization is the conversion of organically bound nitrogen into inorganic mineral forms NH_4^+ and NO_3^-. This process is performed by a wide variety of microorganisms- bacteria, fungi and actinomycetous that utilize nitrogenous organic substances as their energy source. The bulk (95 to 99 %) of soil nitrogen is in organic compounds that protects it from loss but renders it largely unavailable to higher plants. Much of this nitrogen is present as amine group ($R\text{-}NH_2$), largely in proteins or as a part of humic compounds. Microbial decomposition of these compounds produces simple amino compounds, which are hydrolyzed to form NH_4^+ ions. Isotopic tracer studies have shown that mineralized N constitutes a major portion of nitrogen taken up by the crop plants (Brady and Weil, 1998)

The reversal of mineralization is immobilization, the conversion of inorganic mineral nitrogen ions NH_4^+ and NO_3^- into organic forms. As microorganisms decompose carbonaceous organic residue, they may require more N than is contained in the residues themselves. The microorganisms then assimilate mineral N for their growth and maintenance. Immobilization and mineralization are simultaneous process in the soil, working in opposite directions, building up and breaking down soil organic matter respectively (Brady and Weil, 1998). The resulting effect of the two opposing process determines the nitrogen supply to the crop plants (Jansson and Persson, 1982). If the concentration of soil inorganic N increases with time, net mineralization has occurred. On the other hand, net immobilization has occurred when the rate of consumption of inorganic N exceeds the rate of production, resulting in a depletion of soil inorganic N

Despite their importance, both mineralization and immobilization have remained elusive largely because of the difficulty in accurately measuring these processes (Powlson and Barraclough, 1993). The rate of mineralization within the soil profile depends on the distribution of organic substrate and the activity of the microbial biomass. Environmental conditions like soil water content (Nadelhoffer et al., 1991) and temperature (Murphy et al., 1998a; White and Marinakis, 1991) influence the microbial activity within the soil profile and are the most important modifiers of N mineralization. Soil management practices also affects the location and availability of substrate which in turn influences the distribution of microbial biomass, thus altering soil N mineralization (Woods, 1989; Gupta et al., 1994).

The current interest in nutrient cycling in both managed and natural ecosystems has led to renewed attempts to develop reliable methods for measuring N mineralization in the field. This plays a fundamental role in determining the amount of N available to the plants. In arable soils, even where fertilizer applications are made, a substantial part of total N taken up by the plants comes form non-fertilizer source, much of which is derived for mineralization (Powlson, 1988). Improved methods of mineralization measurement could potentially improve fertilizer recommendations by providing more detailed information about the quantity and timing of N release from soil organic matter and help to minimize N losses through nitrate leaching, denitrification or volatilization (Rees et al., 1994).

Mineralization in the soil is quantified in two ways, net mineralization and gross mineralization. The net mineralization can be defined as the result of four different processes: flush effects, basal mineralization, remineralization, and immobilization. The flush effect is caused by changes in soil water and temperature like drying and rewetting or freezing and thawing. It may also result from soil management practices which cause increased exposure of protected organic matter to soil microorganisms (Marumoto et al, 1982; West et al, 1988). The flush is caused by microbial death and subsequent decomposition of microbial cells. The basal mineralization is gross mineralization of soil organic matter which has not been amended with crop residue, and where gross immobilization is negligible. Remineralization occurs after the main decomposition phase, resulting as a recycling of biomass N and microbial death and predation. Remineralization usually consists of a rapid phase, followed by a much slower phase,

therefore, over a short term, only a very small part of inorganic N is recycled (Mary and Recous, 1994; Mary et al., 1993). These process have different kinetics. Flush effects are threshold events, basal mineralization is a continuous process, and immobilization and remineralization vary with the rate and C:N ratio of the residue added to the soil. Similarly, these process respond differently to the environmental fluctuations. This is specially true for soil inorganic N which strongly influences immobilization and remineralization (Fog, 1988). When microbial demand for inorganic N is high, inorganic N is rapidly depleted, and the decomposition rate of organic matter is reduced. As a result, remineralization is delayed but gross mineralization remains unaffected (Hart et al., 1986). The three processes discussed above flush effects, basal mineralization and remineralization constitute gross mineralization. Therefore, net mineralization is the result of the difference of gross mineralization and immobilization.

Numerous methods have been proposed for estimating net mineralization. These include aerobic incubation where soil is leached (Stanford and Smith, 1972) or incubated in closed containers (Kenny, 1982) and anaerobic incubation (Waring and Bremner, 1964). These methods are useful for comparative purposes in that they can rank different soils in terms of their mineralization potential. They have proved useful in studying mineralization kinetics, and understanding how N is released from different organic matter fractions. In ecosystems studies of nutrient flow, the net transformations are of greatest interest, and historically most attempts to measure mineralization have measured the net rates (Rees et al., 1994). This has been partially the result of absence of suitable methods to measure the gross rates. Only a part of mineralized N contributes to the

increase of inorganic pool. The rest is taken up by microrganisms and incorporated again in the soil organic matter. Also, as the size of available N pool is constantly altered by the input-output process of that pool, measurement of individual pool sizes is usually inadequate to quantify individual rate processes. Estimating net mineralization remains essentially an empirical exercise offering little prospect of any detailed understanding of the process governing mineralization-immobilization turnover. Also, validation of empirical models has been reliant on the availability of measurements of gross mineralization and gross immobilization which cannot be measured without using isotopic dilution technique (Murphy et al., 1998b).

Initial work on the concept of measuring gross rates of N mineralization and immobilization by labeling with ^{15}N (Hiltbold et al., 1950) led to the developments of zero and first order analytical models. The N fluxes which enter a N pool or deplete it can be estimated from differential equations describing the rate of change of N and ^{15}N pools and solving these analytically or numerically (Bjarnason, 1988; Myrold and Tiedje, 1986; and Mehran and Tanji, 1974). It has also been demonstrated that adding an NH_4^+ source has a priming effect which gives an overestimate of endogenous N cycling rate process. The principal advantage of isotopic dilution for characterizing microbial processes is that product not a substrate is added to the experimental medium, eliminating the possibility of priming effect (Willison et al., 1998).

The methods of determining gross rates of mineralization and immobilization by measuring the rate of pool dilution of ^{15}N was originally developed by Kirkham and Bartholamew (1954, 1955). Relatively little use of the pool dilution technique was made

during the twenty years following their publications (Smith at el., 1994). However, there have been several recent approaches which involved isotopic dilution techniques to calculate gross mineralization and immobilization in marine sediments (Blackburn, 1979), soil slurries (Ambus et al., 1992), and in paddy soils with and without addition of rice straw (Nisho et al., 1993) using analytical models of zero order.

Applications of these models continued to expand, but the introduction of either different models or different expression of the original model resulted in complications which have not been adequately documented. Isotopic data can be expressed in several forms. For example, Blackburn et al. (1979) used ^{15}N abundance, Barraclough (1991) used ^{15}N enrichment, and Tiedje et al. (1981) used mass of ^{15}N to describe isotopic composition of NH_4 pool. Smith et al. (1994) using the interpretation of "tagged atom" as described by Kirkham and Bartholomew (1954), showed that zero order models of gross mineralization with analytical solutions are of two basic types. Models used by Blackburn et al. (1979), Barraclough et al. (1985) are alternative or equivalent expressions of the original model first proposed by Kirkham and Bartholamew (1954). The second type of the analytical models are based on the arithmetic mean of the data of ratios of labeled and total ammonium pool. These expression were proposed by Bjarnason (1988), Tiedje et al. (1981), and Guiraud et al. (1989).

These analytical models are elegant and relatively easy to derive and use, however, most of the application of these model have traditionally relied on controlled environment, *in vitro* incubation procedures to estimate gross mineralization rates and very few attempts have been made to measure the gross rates in the field. This scarcity of

field measurements is probably because of the cost of ^{15}N analysis, the large number of replicates required to overcome high spatial variability of mineral N, and the considerable time associated with collection and processing of soil samples (Gaunt et al., 1998).

The problem of estimating gross N rates in the laboratory lies in the complexity of organic N turnover, influenced by variable residue and soil characteristics and various soil environmental factors which may not be continuously favorable for biological activity in the field. Consequently, mineralization rates in the field may be lower than those estimated in controlled environments (Wagger et al., 1985). In order to better understand the true extent of nitrogen exchange in the soil plant system it is essential to measure the gross transformation rates in the field. The objectives of this study were: (i) to compare different approaches to estimate N transformation rates; (ii) to compare the effect of previous crop on the gross mineralization and immobilization rates in the field under the winter wheat; and (iii) to evaluate the contribution of legume N to winter wheat grown in the Willamette Valley.

MATERIALS AND METHODS

Experiment:

A long term field experiment was initiated in the fall of 1994 at Hyslop field laboratory near Corvallis, OR. Soil was Woodburn silt loam (Fine-silty mixed, mesic, Aquultic Argixerolls), a moderately well drained soil containing 113 mg kg^{-1} of Bray P, 208 mg kg^{-1} of NH_4OAc extractable K, 9.4 cmole (+) kg^{-1} of Ca, 0.7 cmole (+) kg^{-1} of Mg, and a pH of 6.0 in the surface 0.1 m.

Two cropping sequences: (i) a legume (crimson clover or pea) followed by wheat (*Triticum aestivum* L.), and (ii) oats followed by wheat were established in 96-m by 45-m plots in 1995-96. This cropping sequence remained the same for 1996-97 and 1997-98 growing seasons. Each year four micro plots were established within the 100 kg N ha^{-1} treatments in each rotation by inserting open-ended galvanized sheet metal cylinders into the soil. Each cylinder had a radius of 45 cm, and was manually pushed into the soil to a depth of 20 cm. The micro plots were arranged to include two adjacent rows of wheat. One of the micro plots was fertilized with $^{15}NH_4NO_3$, and another was fertilized with $NH_4{}^{15}NO_3$, while the remaining two were left as unfertilized controls in 1996 and 1997. In 1998 all four micro plots received labeled N fertilizer. Two micro plots received $^{15}NH_4NO_3$ and the other two received $NH_4{}^{15}NO_3$.

The microplot treatments consisted of application of 100 kg N ha^{-1} as a solution of $^{15}NH_4NO_3$ containing 6.811 atom % ^{15}N or $NH_4{}^{15}NO_3$ containing 5.163 atom % ^{15}N in 1996 and 1997, and 5.182 and 5.031 atom % ^{15}N, respectively, in 1998. The fertilizer solution was prepared in the lab by dissolving $^{15}NH_4NO_3$ or $NH_4{}^{15}NO_3$ granules into 250 -

plastic bottles in equivalent amounts to give 100 kg N ha^{-1}. At the time of application the solution was diluted to 1 L in a 1.5 L plastic jar and sprinkled over the micro plots as uniformly as possible. Nitrogen fertilizer was applied to the microplot one day after the large plots were fertilized. The micro plots were covered with a plastic lid while fertilizing large plots.

Plant and soil samples were taken from micro plots at the time of fertilization and again 50 to 70 days after fertilizer application when the crop had accumulated about 1450 to 1615 growing degree days (GDD). The sampling time corresponded to Feekes GS 4 and 8-9, respectively. The plant samples were collected by clipping the rows inside the micro plots about 0.75-cm above the ground. In 1996 and 1997 the plant samples taken at the time of fertilization were from one of the unfertilized controls. The post-fertilization plant sampling included two fertilized micro plots, and the remaining control microplot. In 1998, the two additional fertilized micro plots were sampled after about 25 days of fertilization. Soil samples were collected from micro plots at the same time tissue samples were collected. The soil samples were composites of ten cores collected at 0 to 10 and 10 to 20-cm depths by using a 2-cm soil sampling probe.

Plant samples were dried at 70 ^{0}C in a forced air oven, weighed and ground in Wiley mill to pass 1 mm mesh.. The plant samples were analyzed for total N and C concentration by Leco CNS 2000 combustion analyzer (Leco Corp., St. Joseph, MI) in the Central Analytical Lab of the Department of Crop and Soil Science, Oregon State University Corvallis, OR. The determination of C and N concentration by Leco involved introducing about 0.5 g of tissue into a the combustion chamber. The furnace and oxygen

in the chamber causes the sample to combust. The combustion process converts any elemental carbon and nitrogen into CO_2, N_2 and NO_x. These gases are swept by a carrier gas (helium) to the catalyst heater where NO_x gases are reduced into N_2. Finally these gases are passed through infrared red (IR) cell for determination of C concentration. After removal of CO_2 and H_2O in Lecosorb®/anhydrone tube, the gas is passed through thermal conductivity (TC) cell for determination of N_2 concentration.

For isotopic analysis sub-samples from the ground tissue samples were finely ground (< 75 μm) in acid-washed glass jars, containing six to eight stainless steel bars, on a roller mill for 120 hours. Isotopic analysis for atom % ^{15}N were carried out by CN direct combustion isotope ratio mass spectrophotometer (Europea Scientific., Crewe, England) in the Stable Isotope Research Unit, (SIRU) of the Department of Crop and Soil Science, Oregon State University Corvallis, OR. The isotopic analysis involved introducing about 5-mg of sample into combustion chamber containing catalyst (CrO_3) granules. The combustion products CO_2, N_2, NO_x and H_2O are swept by a carrier gas (helium) into a tube containing Cu wire at 600 ^0C, where NO_x species are reduced to N_2, followed by $Mg(ClO_4)$ and Carbosorb® traps for removal of H_2O and CO_2. The N_2 was purified by gas chromatography, and a small fraction of the effluent was admitted to the mass spectrometer via capillary tubing for measurement of *m/e* 28, 29 and 30, from which both total N and ^{15}N were determined.

Soil samples were air dried and ground to pass 2 mm sieve. Twenty grams of soil samples were extracted with 75-mL of 2M KCl. The extract was the filtered through Whatman No. 42 filter paper. An aliquot of 3.7-mL was pipetted into small plastic vials

for the analysis of ammonium and nitrate nitrogen. The ammonium nitrogen concentration was determined by ALPKEM (Portland, OR) rapid flow autoanalyzer which complexes ammonium with salicylate to form indophenol blue. The color was intensified with sodium nitroprusside and measured at 660 nm. The nitrate nitrogen was determined using the same equipment by reducing nitrate to nitrite via a cadmium reactor and complexing the nitrite with sulfanilamide and N-(1-Napthyl)-ethylenediamine dihydrochloride to form red-purple color measured at 540 nm. Isotopic analysis of soil samples were performed using the same procedure as for plant tissue samples.

Analytical models:

Model I

Estimation of gross rates of nitrogen mineralization using a ^{15}N dilution techniques requires certain assumptions be made to derive a mathematical expression. The main assumptions are: (i) processes such as plant uptake and nitrification do not discriminate between ^{14}N and ^{15}N; (ii) added label mixes with indigenous soil ammonium such that labeled and unlabeled N are exploited equally by ammonium consuming processes; (iii) Over the experimental period, all rate process can be described by zero-order kinetics; and (iv) labeled nitrogen immobilized over the experimental period is not remineralized.

Using the above four assumptions, Barraclough et al, 1985 derived equation [1] for calculating gross mineralization and/or nitrification rates.

$$A_t^* = \frac{A_0}{(1 + \frac{\theta t}{A_0})^{\frac{m}{\theta}}} \qquad [1]$$

where: A is size of ammonium pool, t is time, and m is mineralization rate. Superscripts * indicates atom % excess, subscript 0 and t indicate t = 0 and t = t.

Theta is the rate at which the ammonium pool changes its size.

$$\theta = m - i - n - u - l.$$

where : i , n, u and l are the rates of immobilization, nitrification, plant uptake from ammonium pool and l is the loss of ammonium from the system.

In experimental terms θ is given by:

$$\theta = (A_t - A_0)/t$$

Solving equation [1] for mineralization rate:

$$m = \frac{\theta \times \log(\frac{A_0^*}{A_1^*})}{\log(1 + \frac{\theta t}{A_0})} \qquad [2]$$

The most restrictive assumption for deriving model I is that over the experimental interval immobilized ^{15}N from the mineral pool is not mineralized, in other words there is no remineralization of ^{15}N. This assumption is valid if the experimental interval is short or mineralization-immobilization turnover (MIT) is occurring at a slower rate. However,

The most restrictive assumption for deriving model I is that over the experimental interval immobilized ^{15}N from the mineral pool is not mineralized, in other words there is no remineralization of ^{15}N. This assumption is valid if the experimental interval is short or mineralization-immobilization turnover (MIT) is occurring at a slower rate. However, if MIT is at a faster rate, and the experimental period is long enough, this assumption may not hold true. An expression which eliminates the assumption of no remineralization can be derived using organic N and NH_4 pool.

Model II

Consider two pools, labile organic pool (pool 1) and ammonium pool (pool 2). Let X_i be the amount of total N ($^{14}N + {}^{15}N$) in the ith pool (Fig. 3-1).

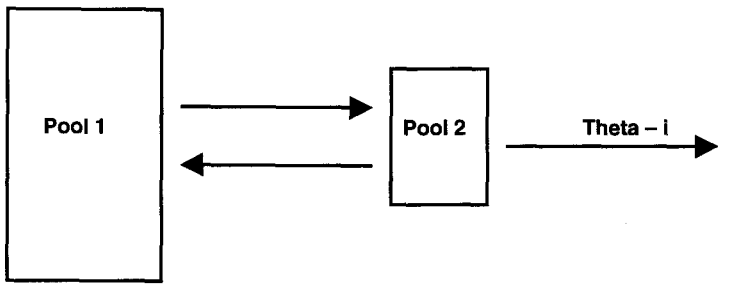

Figure 3-1. A schematic diagram showing rate process of production and consumption of ammonium.

$X = (^{14}N + {}^{15}N)$

Let Y_i be the amount of ^{15}N in the ith pool.

$Y = {}^{15}N$

Let A_i be the atom % excess of ^{15}N in the ith pool, that is:

As the change in size of the ammonium pool with time is a function of the difference between the rate of production (m) and rate of consumption (θ), the change in size of ammonium pool (X_2) with respect to time t can be expressed as:

$$\frac{dX_2}{dt} = m - \theta \qquad [4]$$

And the change in amount of ^{15}N in the NH_4 pool with respect to time t can be expressed as:

$$\frac{dY_2}{dt} = A_1 m - A_2 \theta \qquad [5]$$

Where:

m = rate of mineralization

A_1 and A_2 are atom % ^{15}N excess of pool 1 (labile organic N) and pool 2 (NH_4-N) respectively. Therefore, the change in atom % excess of ^{15}N in the ammonium pool with respect to time is:

$$\frac{dA_2}{dt} = \frac{d(\frac{Y_2}{X_2})}{dt} = \frac{d}{dt}(\frac{Y_2}{X_2}) \qquad [6]$$

$$\frac{dA_2}{dt} = \frac{X_2\frac{dY_2}{dt} - Y_2\frac{dX_2}{dt}}{X_2^2} \qquad [7]$$

By substituting the values from equations [3], [4] and [5] into equation [7]

$$\frac{dA_2}{dt} = \frac{X_2(A_1 m - A_2\theta) - A_2X_2(m - \theta)}{X_2^2} \qquad [8]$$

Simplifying equation [8] and solving for the rate of mineralization

$$m = \frac{dA_2}{dt} \times \frac{X_2}{A_1 - A_2} \qquad [9]$$

Where:

A_2 is atom % excess of ^{15}N of the ammonium pool .

X_2 is the mean size of ammonium pool between t = 0 and t = t.

A_1 and A_2 in the second part of the right hand side of equation [9] are mean atom % excess of ^{15}N between the two sampling intervals (t = 0 and t = t) of both labile organic N and the ammonium pools respectively.

The two basic equations [4] and [5] used to derive expression [9] are same as those used by Tiedje et al. (1981). The advantages of model I over that of model II are

that it is simpler to derive, and most importantly, by using the average ^{15}N excess of the organic pool it takes the remineralization of immobilized ^{15}N into account. Other assumptions in deriving model II are the same as for model I.

To estimate gross mineralization rates, both analytical approaches expressed in model I and model II require that sizes of N pools and the amount of ^{15}N in the pools be measured at the beginning and at the end of the experiment. However, if the measurement of soil N pool and its atom % excess ^{15}N is only made at the beginning of the experiment, gross mineralization can be estimated through the mean pool abundance of mineral N pool using atom % ^{15}N excess of plants.

Model III

Model III involves the concept of estimating mean pool abundance of ammonium or nitrate pool from plant uptake of ^{15}N for calculating the gross mineralization or nitrification rates (Barraclough, 1991). Consider a soil containing 20 kg ha^{-1} as ammonium and the same amount of nitrate. To the soil is added 100 kg ha^{-1} of $^{15}NH_4NO_3$ with 4.366 atom % of ^{15}N in the ammonium moiety. After some interval the crop is harvested and found to contain 2.366 atom % ^{15}N. The proportion of crop nitrogen recovered (N_f) from the NH_4 fertilizer is:

$$N_f = \frac{(A - C)}{(B - C)} \times 100 \qquad [10]$$

Where :

A = Atom % ^{15}N in the plant,

B = Atom % ^{15}N of the fertilizer,

C = natural abundance or the back ground enrichment.

$$\frac{(2.366 - 0.366)}{(4.366 - 0.366)} \times 100 = 50\%$$

Assuming that ^{15}N abundance of a crop that has received no label would be 0.366 atom%. So if the total crop N uptake is 40 kg ha^{-1}, 20 kg N ha^{-1} (0.5 * 40) came from the NH_4 fertilizer. If the crop exploited ^{14}N and ^{15}N of the NH_4 pool in proportion to their relative amounts, then the fraction of ^{14}N and ^{15}N recovered by the crop would be the same. The proportion of added fertilizer NH_4 recovered in the plant was (20/50) x 100 = 40 %, so the amount of native soil NH_4 recovered in the plant is (40/100) x 20 = 8 kg ha^{-1}. Thus, the total recovery of nitrogen from the ammonium pool (N_p) is 20 + 8 = 28 kg ha^{-1}.

We can get the same answer by introducing the idea of mean pool of ^{15}N abundance. For the sake of argument, assume that no mineralization occurred to dilute the label in the ammonium pool following fertilizer application, thus the mean pool abundance over the interval is:

$$[A_{mean}^{*}] = \frac{(x \times C) + (y \times B)}{x + y} \qquad [11]$$

Where:

x and y = the amount of soil and fertilizer NH_4 respectively, substituting the value in equation [11].

$$\frac{(20 \times 0.366) + (50 \times 4.366)}{20 + 50} = 3.2231\, atom\%$$

Now, instead of calculating the recovery of crop N from the added ammonium fertilizer, the recovery of NH_4 from the whole ammonium pool can be calculated by conventional ^{15}N calculation equation [10] by replacing the fertilizer atom % ^{15}N (B) by mean pool abundance [A_{mean}*] and multiplying equation [10] by the crop N uptake (NR_i) equation [12].

$$N_p = NR_i \times \frac{(A - C)}{[A_{mean}^*] - C} \qquad [12]$$

Substituting the values in equation [12]

$$40 \times \frac{(2.366 - 0.366)}{[3.2231] - 0.366} = 28$$

To illustrate this example, it was assumed that the NH_4 pool is not diluted, i.e. mineralization is zero over the experimental interval, however, normally mineralization dilutes the ammonium pool by adding ^{14}N, from the organic pool. Then the mean pool abundance [A_{mean}*] over the experimental period can be expressed by equation [13].

$$[A_{mean}^*] = \frac{1}{\Delta t} \times \frac{A_0^* \times A_0^{\frac{m}{\theta}} \{(A_0 \times \theta t)^{1-\frac{m}{\theta}} - A_o^{1-\frac{m}{\theta}}\}}{\theta - m} \qquad [13]$$

This example illustrates that by measuring the amount of ^{15}N taken up by the plant, total N uptake, and assuming proportional exploitation, the mean pool abundance of the NH_4 pool over the experimental interval can be estimated, which can be used to calculate mineralization or nitrification rate using equation [13].

After estimating the amount of N taken up by the plant from the whole NH_4 pool (fertilizer + soil N) (U_{NH4}) and amount of NH_4 nitrified (N) over the experimental period, net mineralization can be calculated by using equation [14], and immobilization can be calculated as the difference between gross and net mineralization.

$$m_{net} = \theta \times t + U_{NH_4} + N \qquad [14]$$

RESULTS AND DISCUSSION

Soil NH_4 and NO_3 concentration:

Soil ammonium and nitrate-N concentration before and 50 to 70 days after fertilizer application in wheat following clover or wheat following oat in three growing seasons are illustrated in Table 3-1 to 3-3. The NH_4 and NO_3 concentrations did not show any rotation effect in all three seasons at both sampling dates. With the exception of the NH_4 content of pre-fertilization sampling in 1995-96 growing seasons, both NH_4 and NO_3 tested < 5 mg N kg^{-1} at both sampling occasions in the three seasons. Soil NO_3 concentrations were even lower than NH_4 concentrations, and ranged from 0.35 to 2.73 mg kg^{-1} in the three seasons across rotations. Since the soil has a bulk density of 1.3 Mg m^{-3}, 0.35 and 2.73 mg NO_3-N kg^{-1} soil are equivalent to 0.5 and 3.5 kg NO_3 -N ha^{-1}, respectively, in the top 10-cm layer. These concentrations are so low that they did not have any agronomic importance, even though the differences in some cases were tested to be statistically significant.

Plant total N and ^{15}N:

Amount of total N and ^{15}N in the plants at prefertilization and 50 to 70 days after N application in three growing seasons is shown in Table 3-4. During 1997-98, excessive rains just after N fertilizer application caused water ponding in the micro plots, which caused serious losses of the added N through denitrification and/or leaching. As a consequence, nitrogen uptake and amount added ^{15}N recovered in the plants was

considerably lower than for the previous two seasons. Amount of ^{15}N recovered in the plants averaged across labeled N and rotations was only 23 % of the total added in 1997-98 as compared to 84 % and 67 % in 1995-96 and 1996-97, respectively. Mass balance ^{15}N in the plants and soil to the 20-cm depth showed that on average across rotations, 70 % of the added fertilizer remained unaccounted for in 1997-98. Therefore, the data from 1997-98 growing season were not used to estimate N transformation rates.

Table 3-1. Soil ammonium and nitrate-N under winter wheat as influenced by time of sampling, previous crop, and soil depth in 1995-96 growing season.

		Wheat following:			
		Clover		Oat	
GDD†	Depth	NH_4	NO_3	NH_4	NO_3
		---------- mg kg^{-1} ----------			
961					
	0 to10-cm	19.43	2.73	13.08	0.35
	10 to 20-cm	12.46	2.25	9.75	0.46
1455					
	0 to10-cm	4.74	1.83	4.39	0.90
	10 to 20-cm	3.79	2.28	3.64	0.31

		NH_4		NO_3	
Source	df	MS	p-value	MS	p-value
Replication	3	26.03	0.523	0.22	0.783
GDD (a)	1	728.66	0.015	24.76	0.008
Error	3	27.97		0.59	
Rotation (b)	1	45.84	0.204	0.12	0.616
a x b	1	36.77	0.249	0.78	0.220
Error	6	22.56		0.43	
Depth (c)	1	71.70	0.152	0.12	0.478
a x c	1	36.77	0.295	0.09	0.523
b x c	1	7.32	0.634	0.02	0.755
a x b x c	1	5.87	0.669	1.34	0.030
Error	12	30.65		0.22	

† Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 ^{0}C).

Table 3-2. Soil ammonium and nitrate-N under winter wheat as influenced by time of sampling, previous crop, and soil depth in 1996-97 growing season.

		Wheat following:			
		Peas		Oat	
GDD†	Depth	NH_4	NO_3	NH_4	NO_3
		---------- mg kg^{-1} ----------			
999					
	0 to10-cm	3.03	0.58	2.81	0.49
	10 to 20-cm	2.75	0.50	2.66	0.43
1614					
	0 to10-cm	3.91	0.74	3.66	0.75
	10 to 20-cm	3.34	0.58	3.33	0.57

		NH_4		NO_3	
Source	df	MS	p-value	MS	p-value
Replication	3	0.34	0.012	0.42	0.115
GDD (a)	1	4.46	0.000	0.20	0.226
Error	3	0.01		0.09	
Rotation (b)	1	0.16	0.356	0.01	0.513
a x b	1	0.001	0.949	0.02	0.453
Error	6	0.16		0.03	
Depth (c)	1	0.89	0.006	0.12	0.055
a x c	1	0.12	0.251	0.02	0.430
b x c	1	0.07	0.387	0.0001	0.957
a x b x c	1	0.01	0.785	0.001	0.873
Error	12	0.08		0.026	

† Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 ^{0}C).

Table 3-3. Soil ammonium and nitrate-N under winter wheat as influenced by time of sampling, previous crop, and soil depth in 1997-98 growing season.

		Wheat following:			
		Clover		Oat	
GDD†	Depth	NH_4	NO_3	NH_4	NO_3
		---------- mg kg^{-1} ----------			
879					
	0 to10-cm	3.80	1.00	3.58	0.35
	10 to 20-cm	3.67	0.65	3.23	1.50
1509					
	0 to10-cm	4.39	1.50	4.37	1.50
	10 to 20-cm	3.84	1.50	3.73	1.50

		NH_4		NO_3	
Source	df	MS	p-value	MS	p-value
Replication	3	0.35	0.711	0.50	0.500
GDD (a)	1	2.10	0.182	2.05	0.137
Error	3	0.70		0.50	
Rotation (b)	1	0.32	0.468	0.23	0.441
a x b	1	0.15	0.613	0.23	0.441
Error	6	0.53		0.33	
Depth (c)	1	1.40	0.097	1.95	0.049
a x c	1	0.26	0.450	1.95	0.049
b x c	1	0.05	0.733	0.81	0.182
a x b x c	1	0.01	0.895	0.81	0.182
Error	12	0.43		0.41	

† Growing degree days, calculated by taking average daily temperature and taking the cumulative from the date of planting to the date of sampling (base temperature = 0 ^{0}C).

Table 3-4. Nitrogen uptake and atom % ^{15}N of winter wheat grown after legume or oats in three growing seasons.

Growing season	N-form	Time after fertilization	Wheat following: Legume N-uptake	Legume ^{15}N	Oat N-uptake	Oat ^{15}N
		days	kg ha^{-1}	Atom %	kg ha^{-1}	Atom%
1995-96	0-N	0	18	0.374	12	0.370
	$^{15}NH_4NO_3$†	50	140	4.768	85	5.744
	$NH_4{}^{15}NO_3$	50	128	3.839	89	4.638
1996-97						
	0-N	0	32	0.372	6	0.372
	$^{15}NH_4NO_3$	68	128	3.588	68	4.455
	$NH_4{}^{15}NO_3$	68	151	3.488	79	4.163
1997-98						
	0-N	0	56	0.365	22	0.365
	$^{15}NH_4NO_3$	72	68	2.095	51	2.761
	$NH_4{}^{15}NO_3$	72	80	1.641	51	2.070

†Fertilizer was applied at the rate of 100 kg N ha^{-1} in all seasons. The fertilizer contained 6.811 and 5.172 atom % ^{15}N in ammonium and nitrate moiety, respectively in 1995-96 and 1996-97. In 1997-98 the atom % ^{15}N was 5.182 in ammonium labeled case and 5.031 in nitrate labeled case.

Model I vs Model II

Estimation of gross N mineralization rates by isotopic dilution method requires measurement of atom % ^{15}N in the mineral pool at the end of the experimental period. As shown in table 2-1 to 2-3, the NH_4 and NO_3 concentrations were extremely low which made impossible to quantitatively recover NH_4 and NO_3 for isotopic ratio analysis by mass spectrometry. Instead of measuring ^{15}N of the mineral N pool at the end of the experiment, we measured the atom % ^{15}N of the whole soil through direct combustion of soil samples. Whole soil atom % ^{15}N represents the weighted average atom % ^{15}N remaining in both organic and mineral N pools. The atom % ^{15}N excess of the mineral N pool was then estimated assuming distributions of ^{15}N present in the mineral pool ranging from 0 to 100 percent.

One hundred percent ^{15}N in the organic pool would imply that all of the added ^{15}N had accumulated in the organic pool at the time of the second sampling. In contrast, 100 % of the ^{15}N in mineral pool would imply that no immobilization of labeled N had occurred. These assumption represent two extreme conditions, neither of which are probable because of isotopic mixing. Mineralization immobilization turnover (MIT) causes isotopic mixing so that both components, the active soil organic N pool and the mineral N pool have the same ^{15}N content after a given period. For example, in the 1996-97 growing season the amount of ^{15}N recovered from both mineral and organic N pools at the end of the experiment where wheat followed legume was 0.537 kg ha^{-1}. Assuming complete isotopic exchange had occurred in 50 days, the mineral N pool would contain 0.269 kg of ^{15}N and the same amount would be present in the active organic N pool.

The gross mineralization rates estimated using Model I and Model II in the 1995-96 and 1996-97 growing seasons are illustrated in Fig 3-2 and 3-3. These rates were calculated by substituting the size and ^{15}N abundance of the NH_4 pool in equation [1](Model I) and the arithmetic means of two sampling intervals of NH_4 and active organic pools and their ^{15}N abundance in equation [9](Model II). The gross mineralization rates declined with the increase in amount of ^{15}N in the mineral pool, because these models are based on the principle of isotopic dilution of mineral N pool with ^{14}N coming from the organic pool, i.e., the less the inorganic pool is diluted the smaller the mineralization rate. Both models predicted comparable estimates of gross mineralization rates across both rotations in the two growing seasons within the range of 80 to 20 % of the ^{15}N immobilized in the organic pool. Model I is based on the assumption that immobilized ^{15}N is not remineralized whereas Model II corrects for the remineralization of immobilized ^{15}N. As one might expect, the inorganic N pool would be less diluted in a given time if remineralization occurs as compared to no remineralization. Therefore, a model which takes remineralization into account will overestimate mineralization rate if no remineralization is occurring. On the other hand, a model based on no remineralization will underestimate the gross rates if in fact the immobilized labeled N is being remineralized. Model II estimated higher mineralization rates than model I in both rotations and growing seasons within the 80 to 20 % range of ^{15}N in the organic pool. However, the differences between the two models were small and averaged 0.2 and 0.12 kg N ha^{-1} day^{-1} in 1995-96 and 0.05 and 0.05 kg N ha^{-1} day^{-1} in 1996-97 in wheat following clover and wheat following oat, respectively. The similarity

in the two models in predicting the gross mineralization rates within the range of 20 to 80 % ^{15}N in the mineral pool demonstrates that the remineralization during the experimental period was probably negligible. This is supported from the plant N uptake and ^{15}N data (Table 3-4) and from the previous study (Chapter 2) which showed that most of the added ^{15}N was taken up by plants within 30 days of N application, rendering very small amount of labeled N exposed to N cycling processes. Therefore, the immobilization and then remineralization of the remaining ^{15}N was not so remarkable that it could render the assumption of no remineralization completely invalid. However, if the amount of N in the organic pool was > 80 % of the total labeled N, the two models predict very different rates. For example, in the 1995-96 growing season in wheat following clover, Model I and Model II at 98 % of the remaining ^{15}N in the active organic pool estimated 2.6 and 2 kg N ha^{-1} day^{-1} respectively, which is a difference of a total of 30 kg N ha^{-1} over the experimental period. This effect was more pronounced in the first year as compared to the second year, suggesting that the MIT was occurring at slower pace in 1996-97 growing season than in 1995-96.

Gross mineralization rates predicted by both models were higher in wheat following legume than in wheat following oat in the two growing seasons. The difference due to rotation in predicted gross rates tended to increase with the amount of label N in the mineral pool with the effect more apparent in 1996-97 as compared to 1995-96. During 1995-96 the difference in predicted gross rates due to previous crop remained nearly constant at 0.37 kg N ha^{-1} day^{-1}, whereas in 1996-97 the differences due to rotation showed an increased from 0.09 kg N ha^{-1} day^{-1} to 0.18 kg ha^{-1} day^{-1} from 20 to 80 % of

^{15}N in the mineral pool. In both rotations, gross mineralization rates in 1996-97 were approximately one-half of the rates estimated for 1995-96.

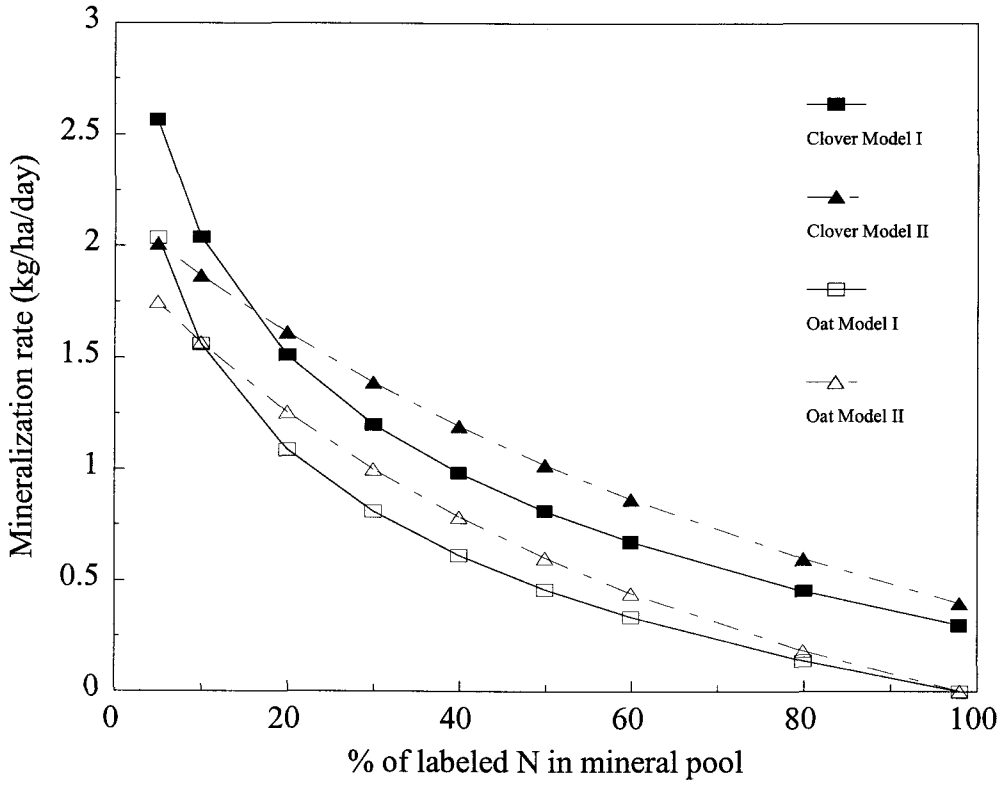

Figure 3-2. Gross N mineralization rates under winter wheat estimated by with and without remineralization models as influenced by previous crop in the 1995-96 growing season.

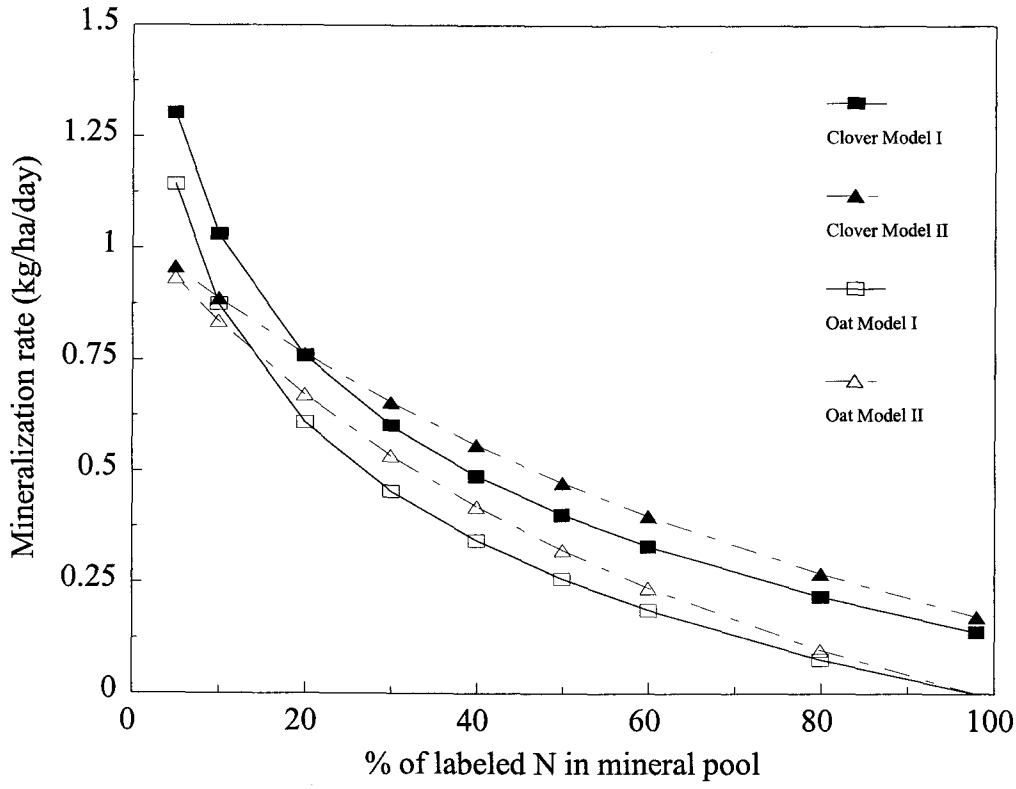

Figure 3-3. Gross N mineralization rates under winter wheat estimated by with and without remineralization models as influenced by previous crop in the 1996-97 growing season.

Model III

In the absence of measured atom % ^{15}N excess for the inorganic N pool, Model III (equation [13]) was used to estimate N transformation rates utilizing the mean pool abundance of the mineral pool. The mean pool abundance of the mineral pool was estimated by equation [12] using the total N uptake over the experiment interval, total N recovered from labeled and unlabeled NH_4 pool and ^{15}N abundance of the plants.

The effect of previous crop on the N transformation rates in the two growing season are illustrated in Table 3-5. Similar to the previous approaches, higher gross mineralization rates were estimated in wheat following clover compared to wheat following oats in both growing seasons. The effect of previous crop was more pronounced in the second year as compared to that of the first year. In 1995-96, the total gross mineralization over the experimental interval in wheat following legume was 1.2 times higher than that of wheat following oats, whereas, in 1996-97 gross N mineralized under wheat following clover was 2.6 times to that of wheat following oat. Higher gross mineralization rates were observed in the first year than in the second year of both rotations, the difference was more pronounced in wheat following oat than in wheat after legume. In 1995-96 the rate of gross mineralization were 2 and 4.3 times greater than in 1996-97 in wheat after legume and wheat after oat respectively, indicating that the oat-wheat system was more N deficient in the second year. This is also evident from net N mineralization calculated by using equation [14]. In both growing seasons the net mineralization was negative in wheat following oat, and became more negative in the second year. In contrast, wheat after legume had 30 and 20 kg more available N than the

wheat after oat over the experiment interval in 1995-96 and 1996-97, respectively. For example, in the first growing season mineralization exceeded immobilization whereas, in the second year both process were occurring almost at the same pace where the legume was grown previously. On the other hand, immobilization in wheat following oat exceeded mineralization in both seasons and was more pronounced in the second year.

Since a pair of micro plots was established, one of which received labeled ammonium and the other labeled nitrate, nitrification was estimated using the same approach of mean pool abundance. Similar to mineralization, nitrification rates in both growing seasons were also higher where the previous crop was legume as compared to oat. However, very low nitrification rates were estimated in both growing seasons and were consistent across the seasons in both rotations. The lower nitrification could be a result of low NH_4 availability to nitrifiers, as the plant competed with them and exhausted the NH_4 pool very rapidly in both seasons.

As it is generally assumed that mineralization in the Willamette Valley peaks in late spring or early summer, it was expected that mineralization immobilization turnover (MIT) would be higher during the experiment, however, our data showed otherwise. Nitrogen mineralization of added crop residue is characterized as a two phasic process following different kinetics. The initial more rapid phase corresponds to the degradation of water soluble amino acid, amino sugars and carbohydrates. The second and slower phase involves the decomposition of cell walls and structural components and the more stabilized products of the first phase. The low mineralization rates in both growing seasons of wheat following clover indicates that the flush effects on N mineralization due

to addition of legume residue had occurred sometime early in the season and during the experimental interval only the decomposition rate of the second slower phase were estimated. This suggests that even though the effect of the legume is evident, wheat crops in the Willamette Valley may not fully benefit from the legume N contribution because of early mineralization and subsequent nitrification and leaching of some of the legume N.

Table 3-5. Gross mineralization and nitrification rates and the total amount of N mineralized, nitrified or immobilized under winter wheat as influenced by previous crop in two growing seasons.

		1995-96		1996-97	
		Wheat following:			
		Legume	Oat	Legume	Oat
Gross mineralization rate	kg ha^{-1} day^{-1}	0.74	0.60	0.37	0.14
Total N mineralized	kg ha^{-1}	36.96	29.77	25.31	9.63
Nitrification rate	kg ha^{-1} day^{-1}	0.23	0.08	0.18	0.05
Total NH_4 nitrified	kg ha^{-1}	11.28	4.11	20.67	3.48
Net mineralization	kg ha^{-1}	20.70	-8.97	0.90	-19.95
Immobilization	kg ha^{-1}	16.26	38.74	24.41	29.58

CONCLUSIONS

Models considering both remineralization and no remineralization estimated comparable gross mineralization rates, indicating that N mineralization immobilization turnover was probably slow in late winter and early spring. All three models estimated higher gross mineralization rates in both seasons where the previous crop was legume as compared to oat. Similarly, nitrification rates were higher in wheat following legume than in wheat following oat. The effect of previous crop on gross mineralization and nitrification rates was more pronounced in the second year. Negative net mineralization showed that fertilizer N was immobilized in the wheat-oat rotation, and the amount of fertilizer N immobilized was greater in the second year. These results imply that wheat after oat was more N deficient in the second year than it was in the first year under the same rotation. Plants depleted soil ammonium and nitrate pools by the time of the second sampling, therefore, it is suggested that if plant uptake is rapid, more than two samplings should be performed. The amount of net mineralization, calculated by estimating gross mineralization and nitrification through the approach of mean pool abundance, was consistent with that observed from the plant N uptake data (chapter 2). This indicates that this approach can give the best approximation of gross mineralization and nitrification rates in such cases where the recovery ^{15}N from the ammonium pool due to plant N uptake is impossible.

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CHAPTER 4

COMPARISONS OF INSTRUMENT CALIBRATION AND TISSUE DIGESTION METHODS FOR DETERMINING ELEMENTAL CONCENTRATION IN PLANTS

INTRODUCTION

Elemental analysis of plant tissue is an integral part of today's modern soil fertility programs. Such analysis of selected leaves or leaf parts serve as an important tool in evaluating fertility status of crop plants, and help soil and crop scientists to formulate more realistic fertilizer programs (Wolf, 1982). With the recent trend to utilize nutrient budgets of agro-ecosystems for the estimation of fertilizer requirements of crop plants, tissue tests are of primary concern to those engaged in monitoring important soil fertility (Haynes, 1980), agronomic, and environmental parameters (Stringari et al., 1996).

During chemical analysis of plant tissue, sample preparation is one of the most critical steps (Rechcigl and Payne, 1990). Phenomenal advances have been made in analytical instrumentation over the last two decades. These include atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry which can analyze large numbers of plant samples in a very short period of time. However, sample preparation techniques have not kept pace with the progress in analytical methods (Nikdell and Temelli, 1987). Transformation of the sample into a form that can be analyzed by a specific measurement technique necessitates destroying organic matter and dissolving plant tissue. This requires a tedious and time consuming procedures like dilution and/or separation and digestion. However, next to plant sampling, digestion of

the sample is the most demanding and error-prone step in the entire analytical procedure (Markert, 1996).

Digestion of plant tissue involves breaking the sample into simple constituents by destroying the organic matrix and converting it into an inorganic one with the aid of time, heat, acid or bases, and catalysts, in open flasks over flames or hot plates, or in other modern digestion vessels (Burguera and Burguera, 1998). Methods of destruction of organic material and dissolution of mineral components in plant tissue are categorized into two main procedures: dry ashing or high temperature dry oxidation and wet acid digestion (Banuelos et al., 1992), frequently referred as dry and wet ashing, respectively (Jones and Case, 1990).

Dry ashing involves heating a sample in an open dish or crucible in air for the combustion of organic matter. The crucibles are often heated in a muffle furnace so as to control the temperature and to maintain a steady flow of air over the sample. The dry ashing procedure requires only simple apparatus and a minimum of working time and is useful particularly in determination of non-volatile constituents of the sample (Bock, 1979). With these advantages of dry ashing procedure, there are a number of fairly severe disadvantages. The chemistry of the dry ashing is very complex, and both oxidizing and reducing conditions may prevail throughout the combustion process. Finally, there are some substances in the sample which are not completely recovered by dry ashing due to incomplete decomposition and volatilization. Jones (1977) reported a dry ashing procedure for use with Inductively Coupled Plasma Spectrophotometer (ICP). He obtained similar concentrations as certified by the National Institute of Standards and

Technology (NIST) for P, K, Ca, and Mg on the Standard Reference Material 1571 (SRM), orchard leaves. However, he observed that the iron concentrations were considerably lower than that reported by NIST (previously National Bureau of standards NBS) due to incomplete digestion of Fe by the dry ashing procedure. Nikdel and Temelli (1987) found that dry ashing of citrus juice in a muffle furnace resulted in considerable volatile losses of B, Cu, K, and Rb. Losses of others easily volatilized elements such as halogens, P, Se, S, Hg, B, Zn and Cu have also been reported during dry ashing (Bock, 1979; Schnug and Haneklaus, 1996).

Wet oxidation involves destruction of organic matter by high temperature acid digestion. Most often, H_2SO_4, HNO_3, and $HClO_4$ are used either separately or with combinations of two or all three. Nitric acid is usually included in most digestion mixture, with the addition of H_2SO_4 to raise the digestion temperature, or the addition of $HClO_4$ or H_2O_2 to speed and complete the digestion (Jones and Case, 1990).

Nitric acid reacts with both aromatic and aliphatic organic material giving rise to oxidation, esterification and nitration reactions. Aliphatic polyhydroxy compounds are particularly susceptible to oxidation by HNO_3, being rapidly degraded to simple carboxylic acids, a factor of considerable significance in the destruction of natural materials which contain many such compounds. Nitric acid boils at 120^0C, a factor which assists in its removal after oxidation.

Perchloric acid is extremely efficient in the destruction of organic material. A mixture of $HClO_4$ and HNO_3 and sometimes H_2SO_4 can destroy majority of organic material. The oxidizing power of $HClO_4$ was first exploited in 19th century in analytical

chemistry through the addition of $KClO_4$ in the Kjeldahl decomposition. Since then numerous procedures have been published for the destruction of organic matter. Though this acid has been used frequently without incident, the occurrence of an occasional explosion of stunning violence has led to its use being somewhat restricted. It is very important, particularly for partly closed systems which do allow evaporation, that a solution containing excess $HClO_4$ should never be brought to complete dryness. Due to its explosive nature, the use of $HClO_4$ is recommended inside specialized fumehoods to provide complete removal of fumes, and a strong shielding of the whole heating apparatus is mandatory to avoid explosion hazard.

Sulfuric acid is the most frequently used component of wet digestion mixtures. The interactions of H_2SO_4 with organic compounds are very complex, however. From the view point of wet digestion, the most important reactions of H_2SO_4 are dehydration and oxidation. In addition to its function in partially degrading organic material by its own action, the presence of sulfuric acid raises the boiling point of the mixture and so enhances the power of other oxidants. The main disadvantage of H_2SO_4 is its tendency to form insoluble compounds and its high boiling point which makes removal of excess H_2SO_4 difficult.

Hydrogen Peroxide has been used for many years as a final treatment to remove small traces of color remaining in the solution after completion of the oxidation process. However, solutions containing 50 % or more H_2O_2 are being used as primary oxidants in association with H_2SO_4. The advantages of using H_2O_2 include its potential as a powerful

oxidizing agent, and moreover the decomposition product left behind after oxidation is only water (Gorsuch, 1970).

The limitations and disadvantages associated with wet digestion include volatility losses, difficulty in digesting large number of samples, use of special equipment, coprecipitation of the element being determined, and explosion hazards (Greenberg et al., 1990). Another limitation of most of the proposed wet digestion techniques is that they usually take several hours to achieve complete digestion of organic material and require constant supervision (Kumar et al., 1997; and Nadkarni, 1984). A significant improvement in conventional wet digestion procedure occurred with the emergence of microwave energy in the analytical field. Microwave digestion provides an alternative to conventional wet digestion methods, and for solid samples it is the most direct way of sample dissolution (Chakraborty et al., 1996). Microwave heating emerged in the field of analytical chemistry in the early 1970s to dramatically speed up or favor some chemical reactions and to improve the digestion process (Burguera and Burguera, 1998). Later, Nadkarni (1984) described microwave application in an open beaker inside a desiccator for acid digestion. The open vessel procedure leads to corrosion and risks of environmental contamination, as well as mechanical and volatile losses of the sample. The open vessel also limits the maximum temperature to the boiling points of acids (Kingston and Jassie, 1986). To over come these limitations, Kingston and Jassie (1986) studied the matter more systemically and used closed Teflon vessels during acid digestion. Pressure digestion in closed vessels lowers the risk of contamination and volatile losses and therefore attracted considerable attention in the field of analytical

chemistry (Bettinelli et al., 1989). The rate of acid digestion in a closed vessel is a function of temperature and pressure. At elevated temperature and pressure much less time is required to reach complete digestion. In addition, compounds that are normally resistant to digestion at the boiling point of acid will react and digest at elevated temperature and pressure (Fischer, 1986). Use of microwave energy with mineral acids like HNO_3 either alone or various combinations with HNO_3, HCl, HF, $HClO_4$ and H_2O_2 have been effective for total sample digestion of various type of matrices and for several elements (Kuss, 1992; White and Douthit, 1985).

Different combinations of acids and different ways of wet digestion have been proposed, however, none of these methods proved to be adequate for complete plant analysis. Anderson and Henderson (1986) used a sealed chamber digestion technique with $HClO_4$ and H_2O_2, and found that digestion of citrus and peach tissues was 96 to 98 % was complete. These authors suggested that the likelihood of $HClO_4$ explosion was decreased considerably due to decreased temperature in the sealed chamber digestion method. On the other hand, they observed large deviations in concentrations between values obtained by the sealed chamber method and those previously established by Everglade Research and Education Center, for Cu, Fe, Mn, Zn, and Al in pecan tissues.

Turner and Brooks (1992) while using H_2SO_4 and H_2O_2 and heat to digest citrus leaves with subsequent analysis on ICP, observed that Ca concentration was significantly lower than the concentration certified by NIST. These workers attributed this to $CaSO_4$ precipitation at higher calcium sample concentrations and suggested that the stability of ICP reading in relation to H_2SO_4 concentration is highly dependent on sample matrix.

Havlin and Soltanpour (1980) observed that HNO_3 and HNO_3 and $HClO_4$ digest resulted in similar concentrations of nine elements in spinach, tomato and orchard leaves, and suggested that HNO_3 is a rapid and precise method for plant tissue analysis. Similar results were obtained by Hung and Schulte (1985) with HNO_3- H_2O_2 and HNO_3 -$HClO_4$ while digesting tomato, corn and alfalfa tissues for determination of 10 different elements. However, the iron concentration from both digestion methods in these plant tissue samples was considerably lower than the concentration certified by NIST. They suggested that HNO_3- H_2O_2 is faster, economical, and safer because concentrated $HClO_4$ is not needed. Contrary to these findings, Zarcinas et al. (1987) found that HNO_3 -$HClO_4$ digestion of orchard leaves SRM 1571 NIST, pea straw, and medic caused loss of K due to sparing solubility of potassium perchlorate, and loss of B due to volatilization. They also observed that iron, sodium and aluminum recovery by using HNO_3 was dependent on type of plant material, and suggested that HNO_3 -$HClO_4$ should be used if these elements are of nutritional interest. In a subsequent study Zarcinas et al. (1996) pointed out that the digestion of peach leaves and pine needles by HNO_3 may be incomplete. Additionally, if the concentration of acid in the final digest does not match with that of standards used for calibration of ICP, inaccurate analysis may result. This is because of variation in droplet size reaching the plasma for the plant digest solution as compared to the standard solution.

No other aspect of plant tissue preparation prior to elemental analysis is as controversial as how to best destroy organic matter portion of the tissue, and it has been difficult to find sufficient fault or advantage with any method that would designate one

superior to another. More importantly, efficacy of a digestion technique, as determined by nutrient analysis, has shown to be dependent on the sample matrix. Also, most of the work on evaluation of a digestion technique has been directed towards horticultural plants, and few studies have looked at comparing different digestion techniques on forage crops. Therefore, this study was carried out with the objectives: (i) to evaluate the effect of different calibration standards commonly used to calibrate the ICP on the determination of elemental concentration in forage crops; (ii) to compare dry ashing, conventional wet ashing and microwave assisted wet ashing as applied to forage tissue; and (iii) to adopt and recommend a technique which will ascertain a reproducible evaluation of mineral status of grass tissue samples.

MATERIALS AND METHODS

Sample collection and preparation

Tall fescue (*Festuca arundinaceae* Sherb) tissue samples were collected from research plots in the Willamatte Valley of Western Oregon. The samples were collected at six dates from late March to early July 1993. Each sample was a composite of three sub-samples. A total of 144 samples were collected.

The samples were dried in forced air oven at 70 ^{0}C for about one week and ground in stainless steel Wiley mill to a fine homogeneous powder to pass a 1-mm mesh screen. All tissue samples were redried at 70 ^{0}C for about 24 hours and placed in a chemical desiccator just prior to weighing and digestion. The samples were digested using three different methods, (i) dry ashing, (ii) HNO_3-$HClO_4$ wet digestion, and (iii) HNO_3-H_2O_2 microwave assisted wet digestion. The digests were analyzed for their concentration of P, K, S, Ca, Mg, Mn, Cu, B and Zn. A National Institute of Sandards and Technology (NIST) reference material orchard leaves (1571) was used as an external control sample.

Dry ashing:

Plant tissues were dry ashed by weighing 0.5-g, weighed to the nearest mg, of sample into a 9-cm quartz digestion tube having an i.d. of 0.6-cm. The quartz tube were placed in a stainless steel rack capable of holding 60 tubes. The rack was placed into a cool muffle furnace (Thermoline Sybron Corporation model F-A1730., Dubuque, IO). The furnace control was adjusted to reach 550 ^{0}C in two hours. After an additional 5.5-

hours of heating the samples were removed and allowed to cool at room temperature for about one hour. Ten mL of 5 % HNO_3 was added to each tube to dissolve the remaining ash, and the aliquot was left to settle over night. Next day the aliquot was transferred into polystyrene round bottom disposable auto sampler tubes for elemental analysis.

The elemental concentration was determined by inductively coupled argon plasma atomic emission spectrometry (ICP-AES) using a Jarrell-Ash Atomscan 9000 (Thermo Jarrell-Ash, Waltham, MA) fitted with cross-flow nebulizer and a cyclonic type cloud chamber with a flow spoiler. The samples were atomized by cross flow nebulization with argon flow rate for aerosol transport of 1-L min^{-1} giving 1.5-mL min^{-1} sample uptake rate, through a 0.045-mm i.d. PVC tube using a 10 roller miniature peristaltic pump (Ranin and Rabbit., Woodburn, MS). Argon coolant flow rate was 15-L min^{-1}, and the auxiliary gas flow rate was 0.2-L min^{-1}. Forward power of 1.1-kW was used to maintain plasma. The optimum viewing zone was 15-mm above the load coil as indicated by height adjustment control on the instrument. Torch extension and 20-L min^{-1} argon purge was maintained for all elements. The wavelengths of the elements determined are illustrated in Table 4-1.

Perchloric ($HClO_4$) Digestion:

One half gram (± 0.001-g) of tissue sample was weighed into a 125-mL Erlenmeyer flask. Twelve mL of concentrated HNO_3 was added to each flask. Prior to acid addition, 5 to 10 glass beads of 1-mm radius were added to prevent bumping during digestion. The flasks were then placed on a 30 x 61-cm model LT-247X3 hot plate

(Sybron-Thermolyne corporation, Dubuque, IA) capable of heating at 370 ^{0}C. The samples were brought to a rapid boil at 120 ^{0}C, and removed after the foaming ceased and the red fumes began to subside. The purpose of this step was to oxidize as much of organic material as possible before adding $HClO_4$. The remaining HNO_3 acts as a diluent for the added $HClO_4$. After cooling the flasks at room temperature for about 20 minutes, 3-mL of $HClO_4$ was added, the flasks were placed back on the hot plate and heated at 125 ^{0}C for about 20 minutes. After the dense white fumes became visible, and the boiling became erratic, the hot plate was turned off and the flasks were left on the plate for 15 minutes. At this point the solution was clear to slightly colored. While the solution was still warm, 10-mL of deionized water was added to each flask immediately after removal from the hot plate and the solution was filtered through Whatman No. 50 filter paper into a 100-mL volumetric flask. The sample flasks were thoroughly rinsed twice with deionized water to ensure complete sample removal. After the sample was completely filtered, 25-mL of hot deionized water was added to each funnel to wash any remains into the volumetric flasks. The volumetric flasks were brought to volume with deionized water after being allowed to cool at room temperature.

The determination of elemental concentration was carried out on a Perkin-Elmer Model 4000 (Perkin-Elmer Corp) atomic absorption spectrometer fitted with a standard premix type air-acetylene burner with a single slot 10-cm burner head. The burner height was adjusted at 6.5 as indicated by height adjustment control on the instrument. The slit width ranged between 0.2 to 0.7-nm , as described by the manufacturer for a given

element. The flow rate of fuel gas (C_2H_2) and air was set at 2.5-L min^{-1} and 10-L min^{-1} respectively, giving a combination of 1:4. The burner head was allowed to warm up for about 5 minutes after ignition. After the flame became uniform and stable throughout its length, samples were aspirated into the plasma through a thin PVC capillary tube, and atomized using an impact bead. Elemental concentration was determined with ordinary mono-or multi element hollow-cathode lamps. The wavelengths of the elements determined are illustrated in Table 4-1.

Table 4-1. Wavelengths of elements determined on Inductively coupled plasma spectrometery and Atomic Absorption spectrometry.

Element	ICP-AES	AAS
	-------- λ (nm) --------	
Phosphorus (P)	178.3	------
Potassium (K)	766.4	766.5
Sulfur (S)	180.7	------
Calcium (Ca)	364.4	422.7
Magnesium (Mg)	279.8	285.2
Manganese (Mn)	257.6	279.5
Copper (Cu)	324.7	------
Boron (B)	259.9	------
Zinc (Zn)	213.9	213.9

Microwave digestion:

Prior to irradiation in the microwave oven, 0.25-g sample of plant tissue was weighed and transferred to 120-mL lined digestion vessels, Teflon® PFA

(perfluoroalkoxy) tubes. Two mL of 30 % H_2O_2 and 0.5-mL of concentrated (70 %) HNO_3 was added to each tube. After gently shaking to make sure that samples were not clumped on one side of the tube, samples were allowed to stand for 10-minutes. The tubes were then fixed into polycarbonate containers, and the containers were capped tightly. The tissue samples were digested using a 640-W CEM microwave oven MDS 2000 (CEM Corporation, Matthews, NC) fitted with pressure monitoring system and exhaust fan. The digestion was carried out at two power and time combinations for a total duration of 12-minutes. The tissue samples were digested at 43 % power for the first four minutes at 75-psi, followed by 82 % power for eight minutes at 200-psi. The microwave unit contained a 360^0 turntable capable of holding 12 teflon vessels, which rotated the samples at three revolutions min^{-1}. After the digestion had been completed, the samples were removed, allowed to cool for 1 hour, and then poured into graduated polystyrene round bottom disposable auto-sampler tubes. The digests were then diluted to 10-ml with deionized water, shaken gently and allowed to settle over night. The elemental concentrations were determined by ICP.

RESULTS AND DISCUSSION

Exploratory data analysis:

Multiple box and whisker plots were constructed for the concentration of each element determined using different calibration and digestion techniques to get a simultaneous visual impression about central tendency, spread or variability, departure from symmetry, tail length and identification of outliers. The side by side box and whisker plots for the concentration of each element are illustrated in Figures 4-1 to 4-9. The box encloses the interquartile range IQR, which is the difference between the 75^{th} and the 25^{th} percentile. The line through the box is at the second quartile, which is the 50^{th} percentile or the median. If the median is in the center of the box the middle portion of the distribution is symmetric. A plus sign in the box shows the sample average. The lines at the either ends of the box (whiskers) extend a distance of 1.5 x IQR. For a standard Gaussian distribution the lower and upper quartiles are at $\mu - 0.6745\sigma$ and $\mu + 0.6745\sigma$, respectively, so the IQR is 1.349σ. Thus 1.5 x IQR is 2.0235σ, and the whiskers extend to $\mu \pm 2.698\sigma$, covering 99.3 % of the population. The relative lengths of whiskers are an indicator of the skewness of the distribution as a whole. The observations beyond the length of whiskers are outliers. A display of parallel box plots can facilitate the comparisons of several batches of data. From the display one can see similarities and differences among the batches with respect to each of five features discussed above (Hoaglin et al, 1983).

The box and whisker plot illustrate that the concentrations of all elements except sulfur were lower when a dry ashed plant sample National Institute of Standards and Technology (NIST orchard leaves 1571) was used to calibrate the ICP (Figs-4-1 to 4-9). Also calibrating the ICP with a dry ashed plant standard (NIST 1571) for the analysis of dry ashed samples resulted into a smaller spreads than the chemical standard calibration. Samples for the study were collected in such a way that they represented a wide range of concentration of these elements, therefore the spread of the data set has a different meaning rather than a measure of precision. In other words, a smaller spread is an indication of loss of an element during a digestion process, while a larger spread shows the opposite.

The characteristics of data for the concentration of nine elements displays that the distributions of data obtained by various techniques are almost symmetrical around the center. However, slight skewness can also be seen in Mg, S, B, Cu and Zn concentrations obtained from microwave assisted and perchloric acid digested samples, irrespective of the standard used for calibrating the equipment.

The data sets for P and Mg concentration (Fig 4-1 and 4-4) illustrate that the spreads of different digestion and calibration techniques are almost identical, with the exception of dry-ashed samples determined by calibrating the ICP with a dry-ashed standard. The data have a smaller spread with a few outliers on the higher end of the distribution. The data set for potassium concentration (Fig 4-2) shows slightly bigger spread of perchloric acid digest samples than that of other methods. The calcium concentration (Fig 4-3) showed more variation in microwave digested samples than the

other methods. The distribution of sulfur concentration (Fig 4-5) in dry ashed samples using chemical standard to calibrate ICP resulted in a distribution having a shorter tail than the other methods. Microwave digest samples showed more variability than the dry ashed samples. The concentration of boron (Fig 4-6) in dry ashed samples with dry-ashed standards produced a somewhat skewed distribution towards high concentration. The concentration of boron determined in microwave digest samples, using microwave digest standard showed more variability as compared to the other methods. The data set for copper concentration (Fig 4-7) resulted almost similar distributions of microwave digested and perchloric acid digested samples but were more dispersed than the distribution of dry ashed samples. On the other hand, the distributions of dry ashed samples differed in spread with the standard used for calibration. The distribution of manganese and zinc concentration (Fig-4-8 and 4-9) shows that microwave digestion resulted in a larger spread than that of dry ashing. The distribution of Zn concentration of microwave digested samples also has a few outliers in the upper end of the distribution. Also the microwave digestion yielded higher concentration than the perchloric acid digestion when the ICP was calibrated with a chemical standard.

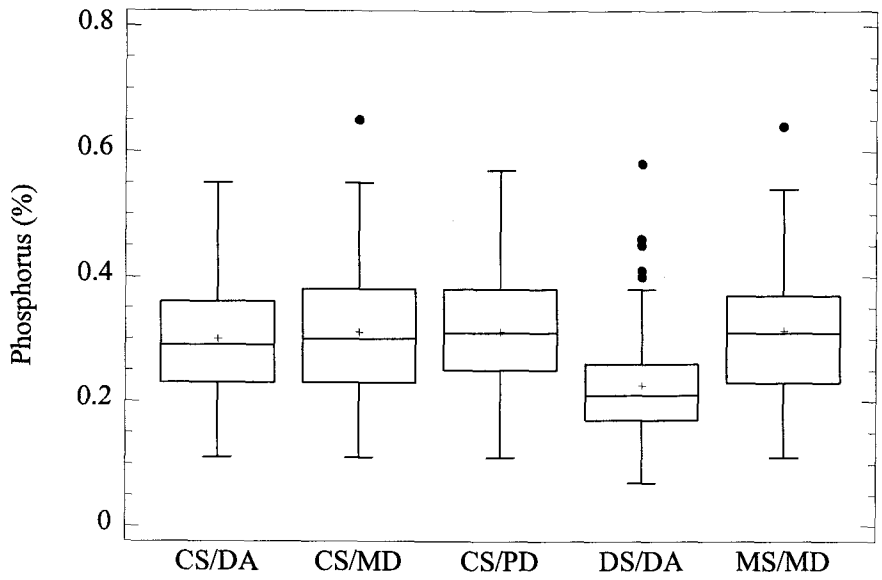

Figure 4-1. Box and whisker plots for P concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

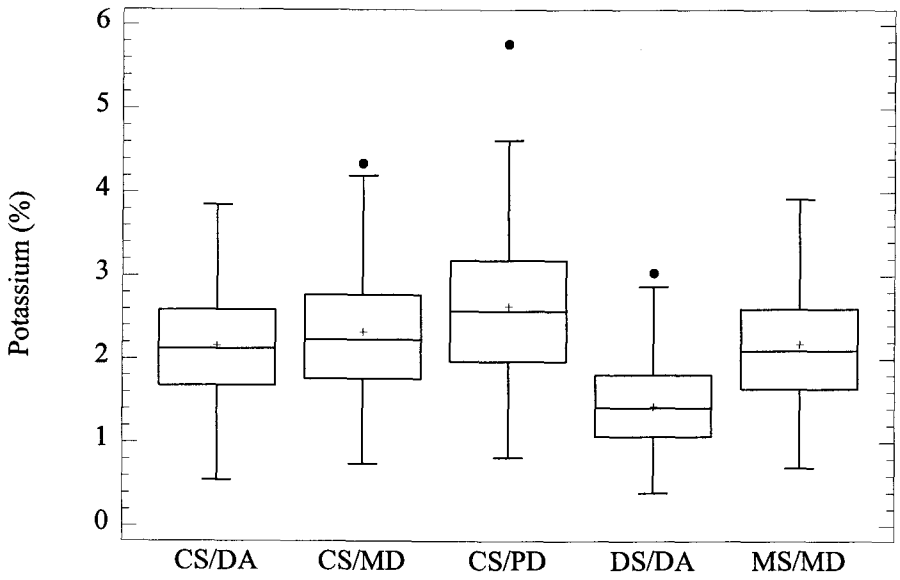

Figure 4-2. Box and whisker plots for K concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

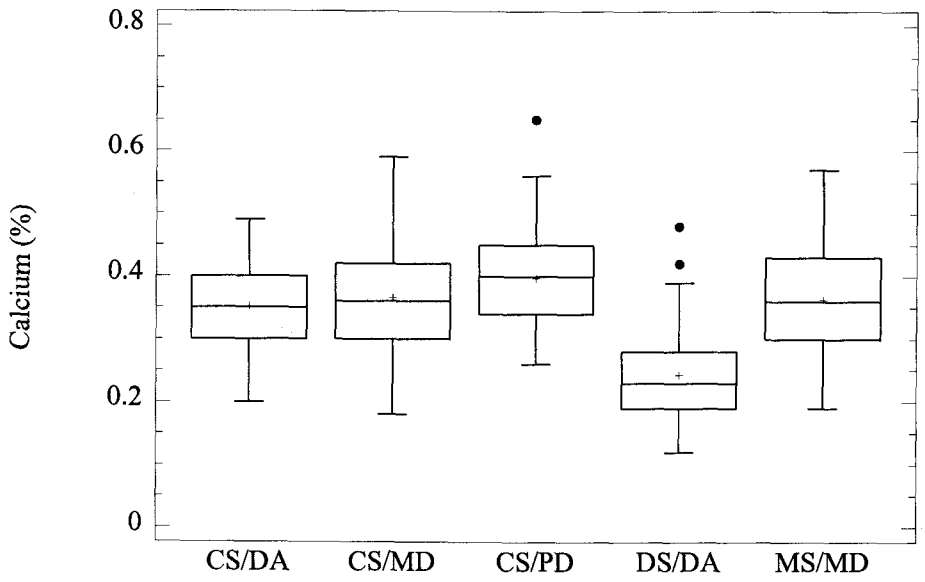

Figure 4-3. Box and whisker plots for Ca concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

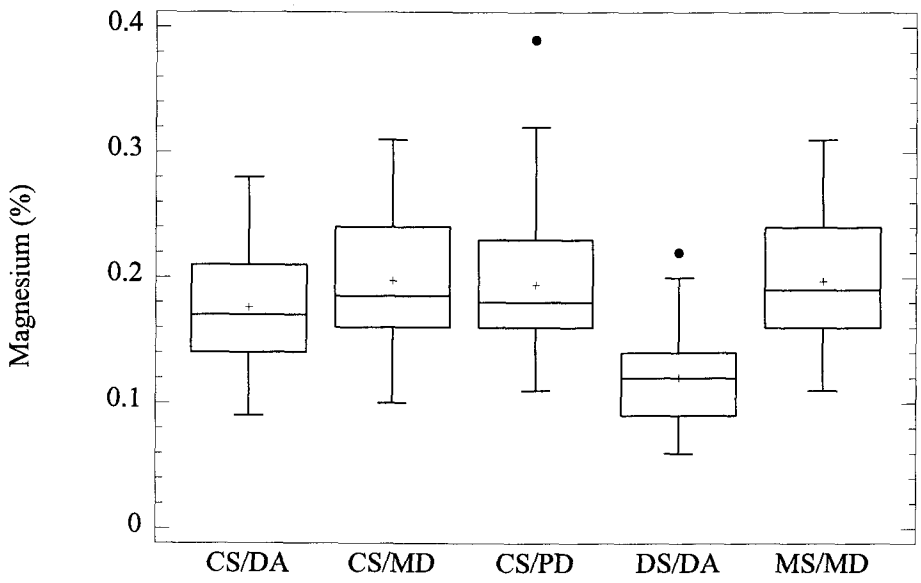

Figure 4-4. Box and whisker plots for Mg concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

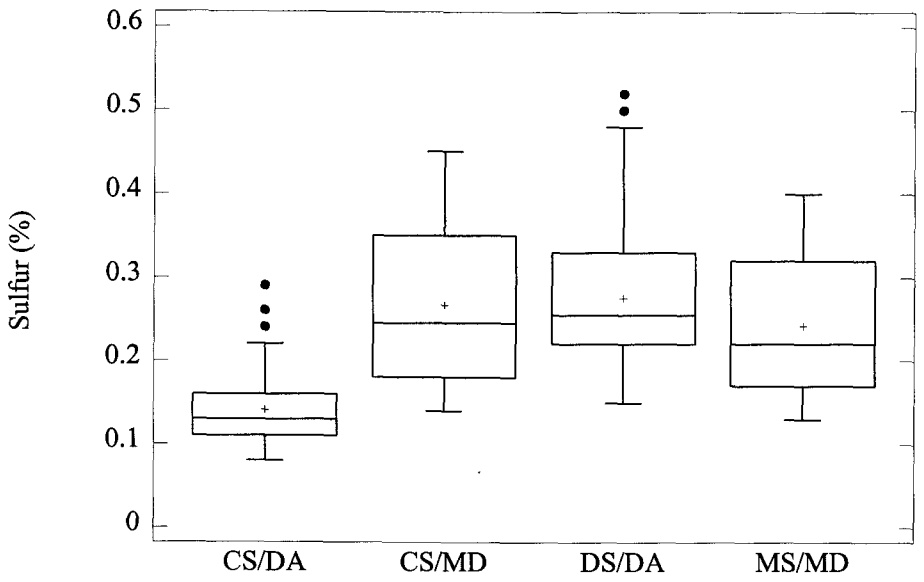

Figure 4-5. Box and whisker plots for S concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

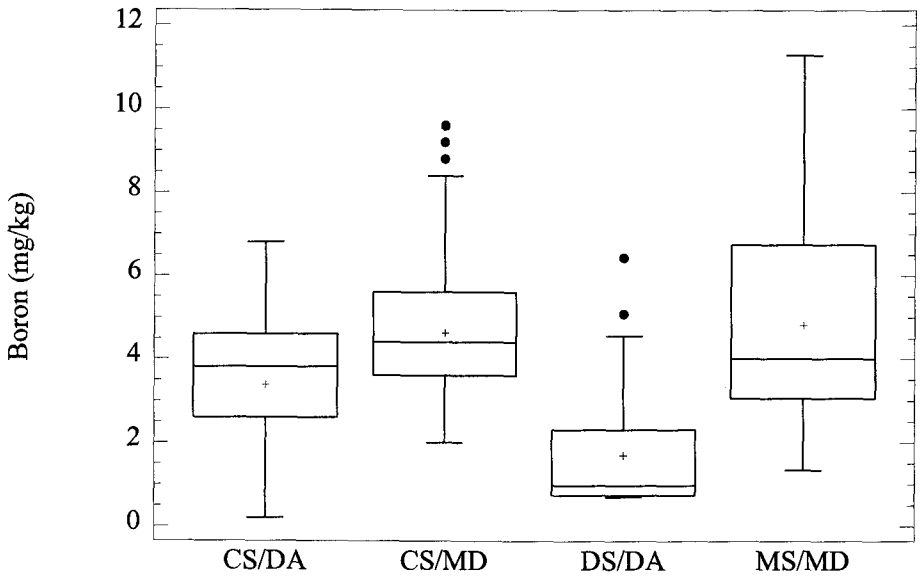

Figure 4-6. Box and whisker plots for B concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

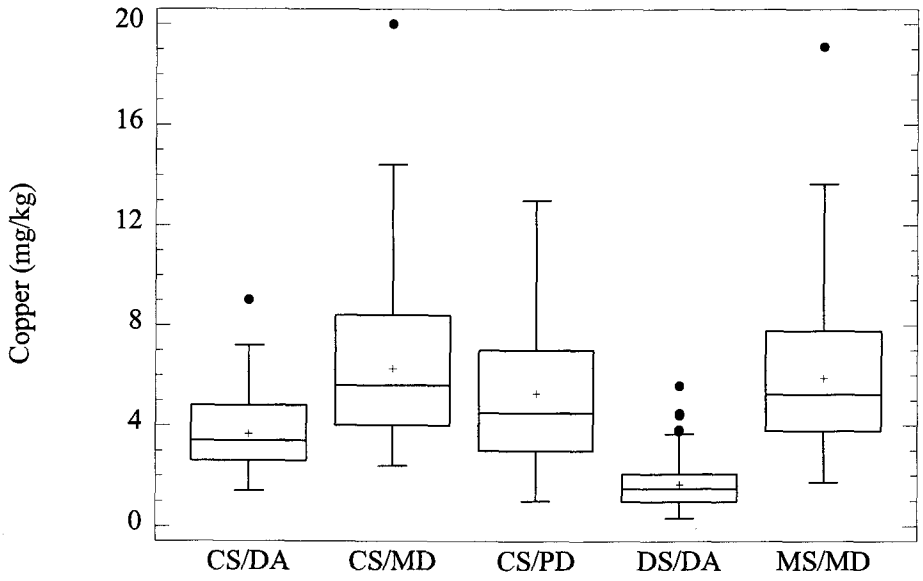

Figure 4-7. Box and whisker plots for Cu concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

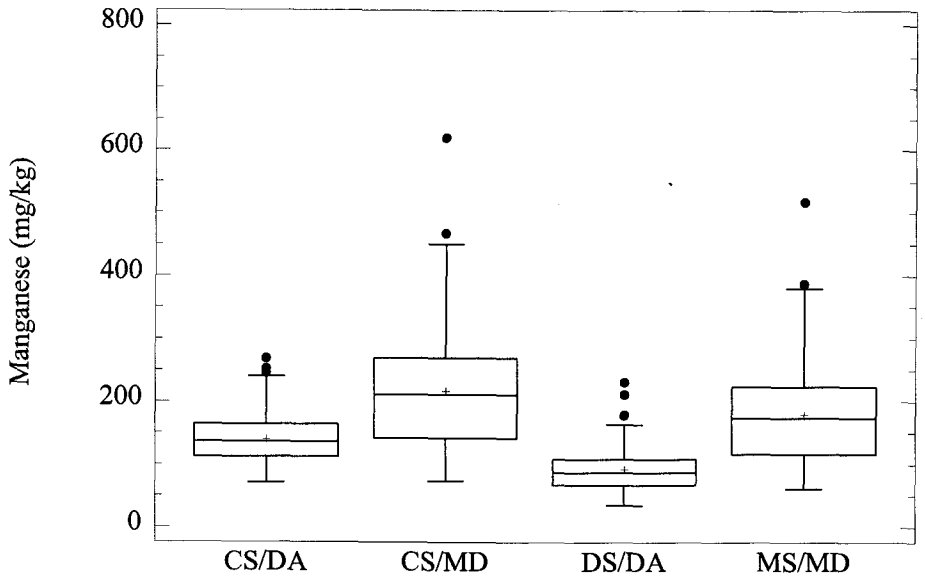

Figure. 4-8. Box and whisker plots for Mn concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

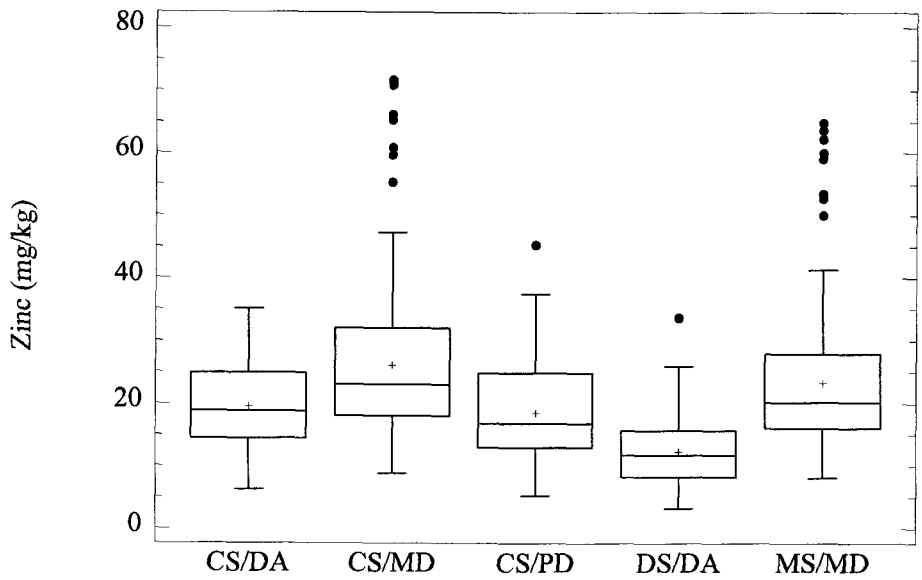

Figure 4-9. Box and whisker plots for Zn concentration obtained by different calibration and digestion techniques. DA = Dry-ashed sample, DS = Dry ashed standard, MD = Microwave digestion, CS = Chemical standard, MS = Microwave digest standard, and PD = Perchloric acid digest. The box includes the interquartile range (IQR) the 25th and 75th percentile, the whiskers extend to 1.5 IQR, the vertical line inside the box is median and the plus sign is the sample mean.

Equipment calibration:

Dry ashed versus Chemical standard:

Regression analyses were performed to compare different standards for ICP calibration, and to determine if there is a linear association between different standardization techniques. Least square method was used by regressing one standardization method on another. Figures 4-10 and 4-12 show the scatter plots and the fitted regression lines of the concentration of nine elements determined using either calibrating the ICP with a chemical or a dry ashed standard.

In general, regression analysis showed higher concentration when the ICP was calibrated with chemical standard as compared to dry-ashed standards. Concentration of S, B, Ca, Mg, Mn, Cu, (Fig 4-10bc, 4-11bc and 4-12ab) in the dry ashed samples obtained by calibrating the ICP with dry ashed standard showed either no or very weak correlation with the concentration obtained by calibrating the ICP with a chemical standard. The data points showed a random scatter along the regression line. The correlation coefficient ranged between 0.77 to 0.48. Only in case of P, K and Zn (Fig 4-10a, 4-11a and 4-13c) was a linear relationship observed between the dry ashed and chemical standard calibration, but again the relationship was not very strong. The correlation coefficient between the two calibration techniques ranged between 0.82 to 0.83.

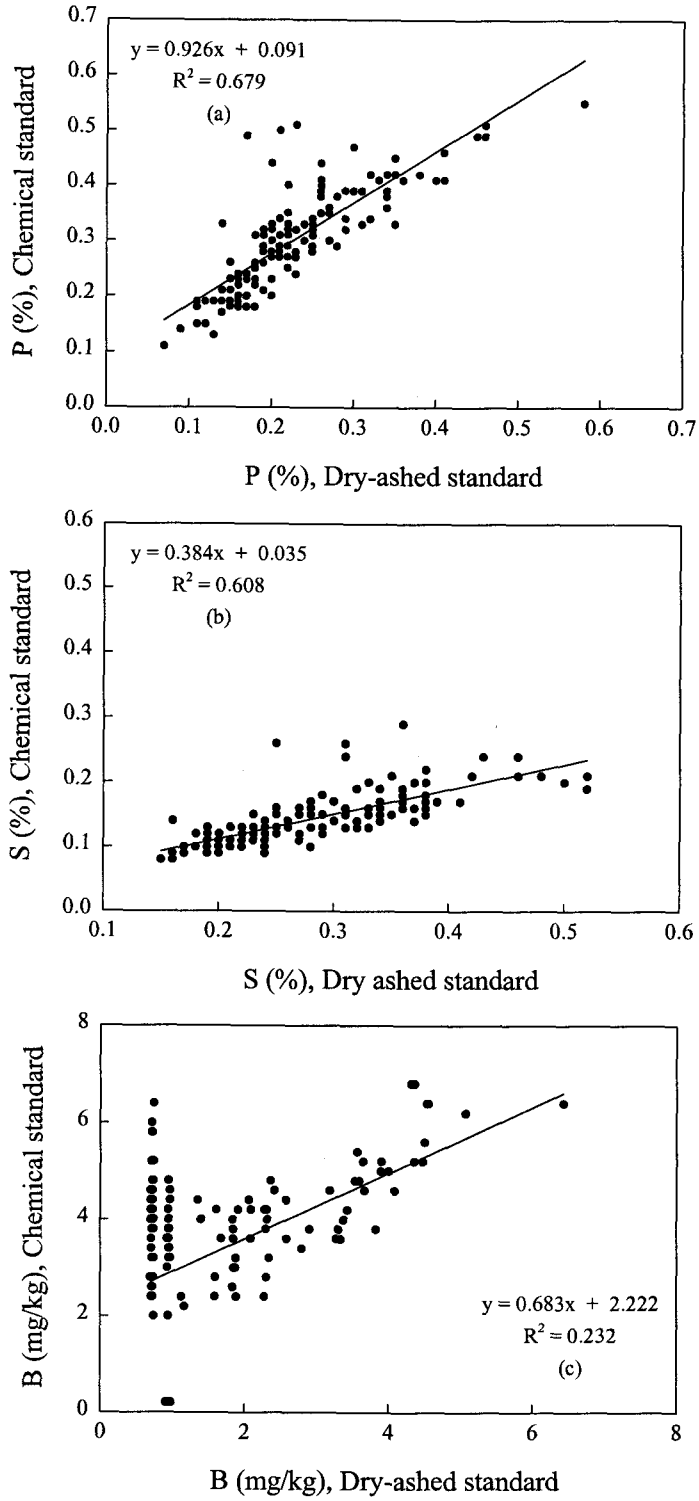

Figure 4-10. Comparisons of ICP calibration with chemical standard or dry ashed standard for phosphorus, sulfur and boron.

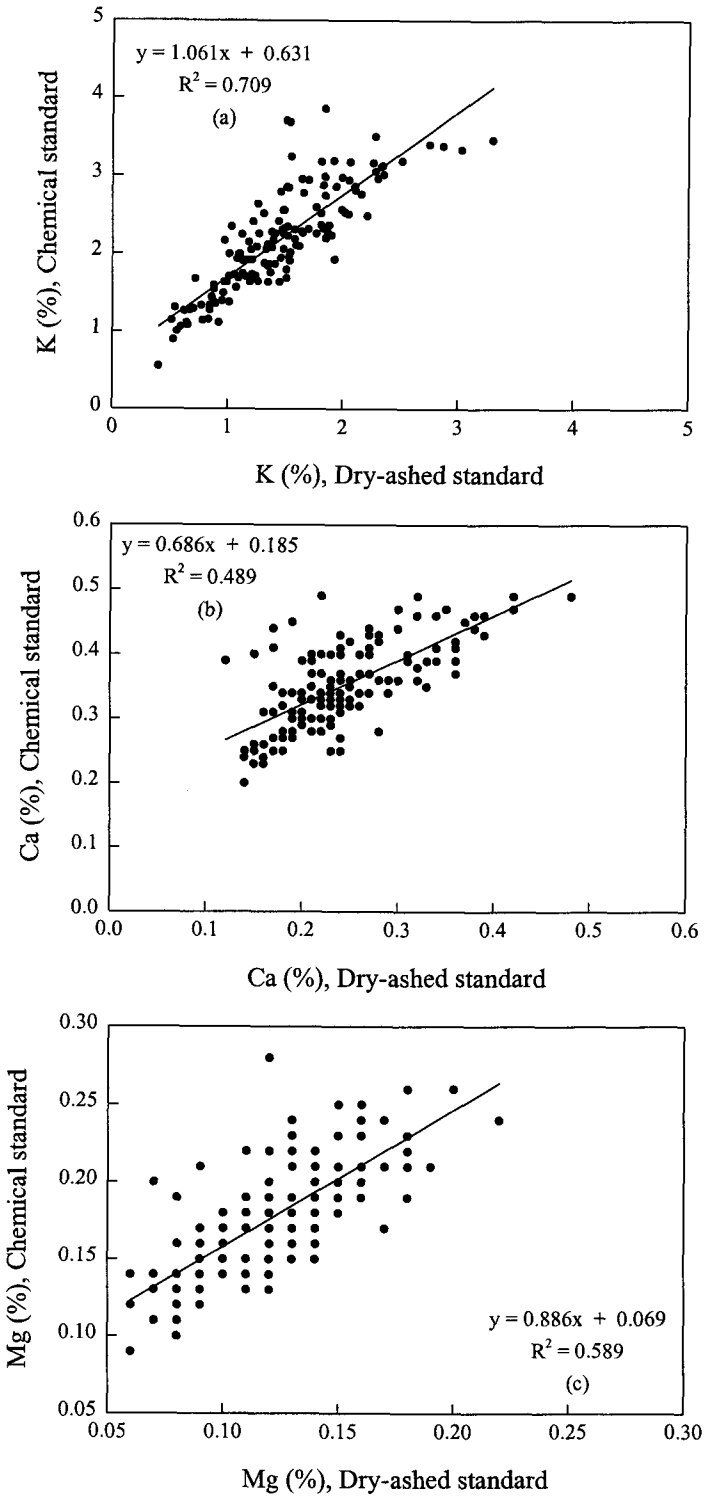

Figure 4-11. Comparisons of ICP calibration with chemical standard or dry ashed standard for potassium, calcium and magnesium.

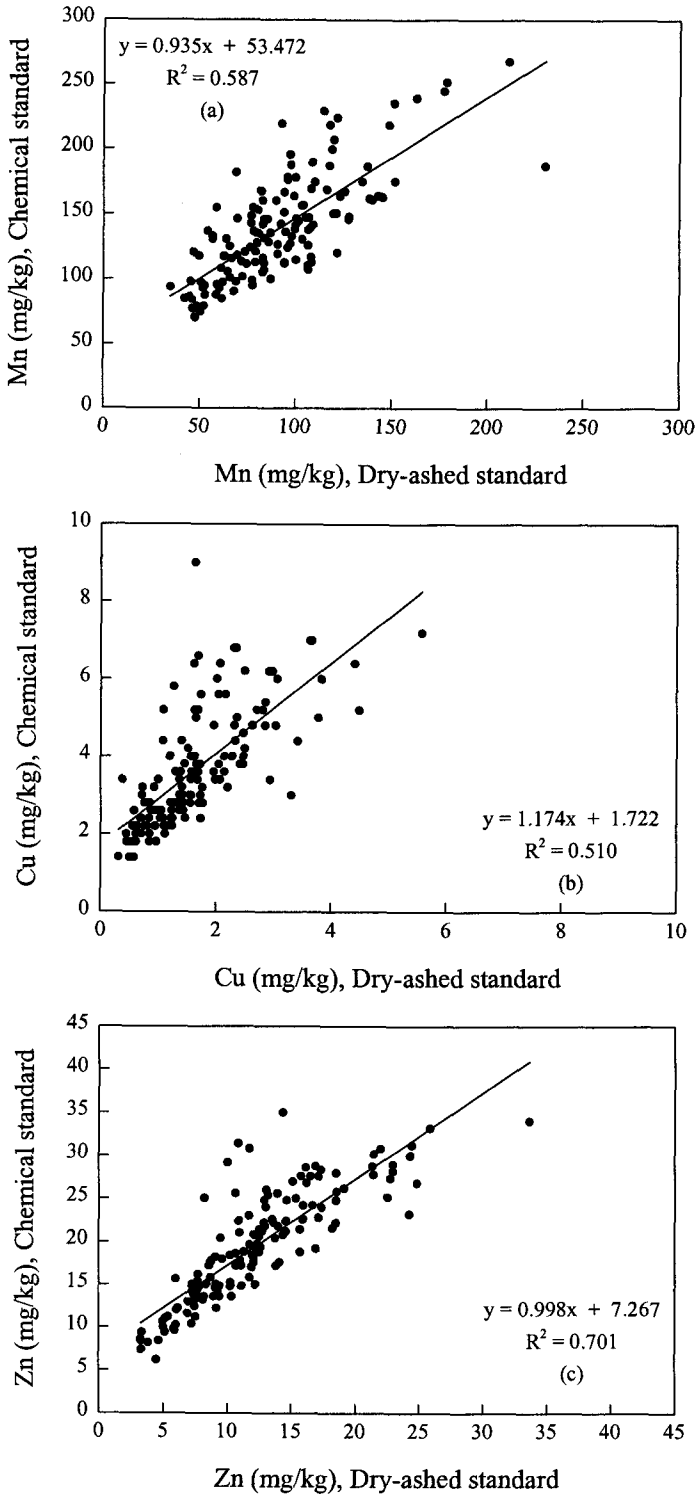

Figure 4-12. Comparisons of ICP calibration with chemical standard or dry ashed standard for manganese, copper and zinc.

The elemental analysis means with variances for two calibration techniques (dry ashed standard and chemical standard calibration) for the dry ashed tall fescue samples are summarized in Table 4-2. Similar to the comparisons made by box and whisker plots, which showed that the spread and medians were smaller for all elements except S when the ICP was calibrated using dry ashed standard as compared to chemical standard, the comparisons of means showed that with the exception of S, calibration using chemical standard yielded significantly higher elemental concentrations than did calibration with dry ashed standard. When averaged across elements, the concentration obtained using chemical standard to calibrate ICP was 1.6 times higher than that obtained by using dry ashed standard. In general, there appeared to be a difference of higher magnitude between the calibration techniques for the micronutrients Mn, Cu, B and Zn, whereas the magnitude of difference in macronutrients was relatively small. The difference for micronutrients was 1.3 times greater than that observed for macronutrients between the two calibration techniques. This is probably because the concentration of micro elements are substantially lower, which affects the measurements capability of the ICP. A similar pattern was observed for variances, with the exception of sulfur, the calibration with chemical standard showed bigger variances than that of dry ashed calibration. However, the differences were not significant for P, Ca, and Mg.

The precision and accuracy values for the concentration of these elements will have two components-one associated with standard preparation (dry ashed or chemically prepared) and the other associated with the analytical performance of the equipment. As dry ashing the standard could have caused varying volatile losses of these elements, it is

more likely that the differences in the concentration values were largely due to standard preparation involving losses and the capability of acid to bring elements in the solution after dry ashing and not due to the influence of chemical and physical properties of standard on the analytical performance of ICP.

Table 4-2. Elemental concentration in dry ashed samples of Tall fescue as influenced by calibrating the equipment with either dry ashed or chemical standards.

	Dry-ashed standard		Chemical standard		Significance‡	
Element	Mean†	S^2	Mean	S^2	Means	S^2
P§	0.226	0.0071	0.300	0.0090	0.0000	NS
K	1.443	0.2884	2.163	0.4578	0.0000	0.0061
S	0.276	0.0067	0.141	0.0016	0.0000	0.0000
Ca	0.244	0.0047	0.350	0.0045	0.0000	NS
Mg	0.121	0.0012	0.176	0.0015	0.0000	NS
Mn	92	1104	139	1641	0.0000	0.0496
Cu	1.653	0.8360	3.662	2.2577	0.0000	0.0000
B	1.698	1.5885	3.378	3.1074	0.0000	0.0002
Zn	12.297	30.076	19.512	42.917	0.0000	0.0298

†Means and variances for n = 142. ‡The significance of the differences between the two means was tested by paired t-Test, whereas the significance of the two variances was tested by Levene's Test for homogeneity using two sample t-Test. §P, K, S, Ca and Mg are expressed in %, the remaining elements are expressed as mg kg^{-1} (dry mass basis).

Concentration values of the samples are a function of the slope of a curve which is generated by the ICP when the ICP is calibrated using some standard. A calibration curve with a steeper slope will yield low concentration at the same emission intensity than the

one with a relatively gradual slope. Although it is evident that a steeper calibration curves was generated by the ICP with a dry ashed standard than with a chemical standard for all elements except sulfur, the reverse might also be expected due to unpredicted elemental composition of dry ashed standard. This could be explained by comparing S and Mn concentration, as both of these elements are prone to volatilization due to high temperature therefore a dry ashed standard should generate a steeper curve than a chemical standard for both elements. The calibration curve generated for S showed an opposite pattern of that generated for Mn. In other words the calibration curve generated with dry ashed standard for Mn concentration was steeper than the curve generated by chemical standard, whereas in case of S the opposite was true. As no S losses occurred during chemical standard preparation, this suggests that a dry ashed standard could generate such a highly variable calibration curve, that is not a good predictor of sample concentration.

Chemical standard versus Microwave digested standard

Table 4-3 presents comparisons between calibrating the equipment with chemical standard and microwave digested standard for the concentration of 9 elements in tall fescue microwave digested samples. Consistent with the comparisons showed by box and whisker plots between the two standards, concentrations obtained by calibrating the ICP with a microwave digested standard were comparable to those obtained by using the chemical standard. Concentration ratios of chemical standard to microwave digested

standard averaged 1.1. The differences due to calibration were not significant for Ca, Mg and B. It should be mentioned that with the exception of Mn and Zn, the differences although statistically significant for P, K, S, and Cu are quite acceptable for diagnostic purpose.

Compared to dry ashed standard the differences between chemical and microwave digested standard calibration were small, and both calibration techniques showed almost same variances for all elements except S and B. Though the reddish fumes released were mainly of nitric acid as the lids of teflon tube were unscrewed after microwave digestion was complete, these fume might have contained some S and B. Therefore, the smaller variances of S and B were probably due to the volatility of these elements after microwave digestion. Also, the differences of micronutrient concentration in the two calibration technique were not as big as observed in dry ashed calibration, suggesting relatively small losses and thereby less variability in microwave digestion than in dry ashing. The calibration curves generated by the ICP using the two standards were almost the same for Ca, Mg, and B and were not remarkably different to each other for P, K, S, and Cu. Therefore, if the nutrients of interest are not Mn or Zn either calibration technique can be used, as many labs because of relatively high cost of Standard Reference Materials (SRMs) or chemicals standards, use their own home developed plant tissue standards.

Acid in microwave digestion functions as an oxidizer and its concentration in digested sample may vary depending upon the sample matrix. The difference in acid

Table 4-3. Effect of microwave digested standard and chemical standard used for calibrating the equipment for determination of elemental concentration in tall fescue.

	Chemical standard		Microwave digest standard		Significance‡	
Element	Mean†	S^2	Mean	S^2	Means	S^2
P§	0.311	0.0114	0.314	0.0115	0.0078	NS
K	2.325	0.5970	2.179	0.5145	0.0000	NS
S	0.267	0.0081	0.242	0.0065	0.0000	0.0456
Ca	0.365	0.0071	0.363	0.0066	NS	NS
Mg	0.198	0.0025	0.196	0.0023	NS	NS
Mn	216	9124	179	6146	0.0000	NS
Cu	6.259	9.1057	5.893	8.1444	0.0000	NS
B	4.600	3.0837	4.623	7.9847	NS	0.0001
Zn	26.00	151.64	23.268	119.76	0.0000	NS

†Means and variances for n = 142. ‡The significance of the differences between the two means was tested by paired t-Test, whereas the significance of the two variances was tested by Levene's Test for homogeneity using two sample t-Test. §P, K, S, Ca and Mg are expressed in %, the remaining elements are expressed as mg kg^{-1} (dry mass basis).

concentration between sample and standard can effect nebulizer performance and thereby plasma temperature.

One advantage of using microwave digested standard for ICP calibration for analyses of microwave digested samples is that by matching the matrix of the standard with that of the sample, matrix effects could be minimized, provided that the standard and sample tissue are the same. Although, in this study the SRM was orchard leaves which had a different matrix than the grass samples, the results of both calibration techniques showed a good agreement. However, considering losses and variation caused by

digestion, the effect of tissue matrix on spectral and non-spectral parameters of ICP, and the simultaneous determination of these elements, which is a conventional practice of most of the plant analyses lab, it is suggested that using chemical standard for calibration is a more reliable technique than using either dry or wet ashed standards.

Plant tissue digestion:

Microwave assisted digestion versus dry ashing:

Comparisons for the concentration of nine elements obtained by either dry ashing or microwave assisted digestion are illustrated in Table 4-4. These concentration values were obtained by calibrating the equipment with chemical standard. The box and whisker plots indicated that the median concentrations and the spreads for P, K, Ca, Mg, were comparable, whereas for S, Cu, Mn and Zn, these two parameters differed considerably between microwave digestion and dry ashing. Whereas median B concentration differed in the two digestion methods but the spread was almost identical. Similar to the exploratory data analysis, the differences in elemental concentrations between the two digestion techniques, though significant, were small for P, K, Ca and Mg, the ratio of microwave digestion to dry ashing means averaged 1.1. On the other hand, concentration of S, Mn, B, and Zn showed greater differences between the two digestion methods. Sulfur concentration was almost twice as high in microwave digested sample as that of dry ashed, whereas, the concentrations of Mn, Cu, B and Zn were, on the average, 1.6 times higher in microwave digest samples than that of dry ashed. The differences in variances between the two digestion techniques for P, K and B were not significant, and

were small for Ca and Mg. In contrast, the variances of the concentrations for S, Mn, Cu and Zn were about 3 times bigger for microwave digested samples than that of dry ashed, indicating that the losses of these elemental were greater in dry ashing as compared to microwave digestion.

Table 4-4. Elemental concentration of tall fescue as influenced by dry ashing and microwave digestion techniques, using chemical standard to calibrate the equipment.

	Microwave digest		Dry ashed		Significance‡	
Element	Mean†	S^2	Mean	S^2	Means	S^2
P§	0.311	0.0114	0.300	0.0090	0.0000	NS
K	2.325	0.5970	2.163	0.4578	0.0000	NS
S	0.267	0.0081	0.141	0.0016	0.0000	0.0000
Ca	0.365	0.0071	0.350	0.0045	0.0000	0.0024
Mg	0.198	0.0025	0.176	0.0015	0.0000	0.0008
Mn	216	9124	139	1641	0.0000	0.0000
Cu	6.259	9.1057	3.662	2.2577	0.0000	0.0000
B	4.600	3.0837	3.378	3.1074	0.0000	NS
Zn	26.00	151.64	19.512	42.917	0.0000	0.0006

†Means and variances for n = 142. ‡The significance of the differences between the two means was tested by paired t-Test, whereas the significance of the two variances was tested by Levene's Test for homogeneity using two sample t-Test. §P, K, S, Ca and Mg are expressed in %, the remaining elements are expressed as mg kg^{-1} (dry mass basis).

Microwave assisted digestion versus perchloric acid digestion:

Comparison between mean P, K, Ca, Mg, Cu and Zn concentrations obtained by microwave digestion or perchloric acid digestion are presented in Table 4-5. The comparisons of the two methods by box and whisker plots indicated that both of digestion techniques yielded comparable medians and variances for the concentrations of all elements except Zinc. Similarly the comparisons of means showed that the concentration of P and Mg were identical in both digestion methods. Though the differences were significant for K, Ca, and Cu, the magnitude of differences was very small and the ratio of the two digestion techniques averaged 1.

Table 4-5. Effect of microwave assisted and perchloric acid digestion techniques on determination of elemental concentration of tall fescue.

	Microwave digest		Perchloric acid digest		Significance‡	
Element	Mean†	S^2	Mean	S^2	Means	S^2
P§	0.311	0.0114	0.312	0.0095	NS	NS
K	2.325	0.5970	2.633	0.6812	0.0000	NS
Ca	0.365	0.0071	0.397	0.0055	0.0000	NS
Mg	0.198	0.0025	0.194	0.0021	NS	NS
Cu	6.259	9.1057	5.283	7.3834	0.0001	NS
Zn	26.002	151.640	18.594	58.656	0.0000	0.0037

†Means and variances for n = 142. ‡The significance of the differences between the two means was tested by paired t-Test, whereas the significance of the two variances was tested by Levene's Test for homogeneity using two sample t-Test. §P, K, S, Ca and Mg are expressed in %, the remaining elements are expressed as mg kg^{-1} (dry mass basis).

With the exception of Zn, both digestion techniques showed an excellent agreement between the mean concentrations of all elements. Both the concentration and variance of Zn was higher in the microwave digested samples than by perchloric acid digestion. The variance was almost three times higher in microwave digested samples as compared to those digested by perchloric acid. As the Zn concentration obtained by perchloric acid digestion was comparable to that obtained by dry ashing, and perchloric acid digestion showed smaller variance than microwave digestion, the data suggest that Zn was lost during perchloric acid digestion as well. In contrast to dry ashing, the differences between microwave and perchloric acid digestion methods for Cu concentration were also minimal, suggesting lower Cu losses in perchloric acid digestion than in dry ashing. In general, the data suggest that both digestion techniques are almost equally effective for the determination of elemental concentration in grass samples for routine diagnostic purposes.

Comparisons of different digestion technique with chemical standard calibration for 9 elements using regression analysis are presented in Fig- 4-13 through 4-19. A close association was observed between the P concentration obtained by microwave digestion and dry ashing the samples, when the equipment was calibrated with chemical standards in both cases (correlation coefficient = 0.97) (Fig 4-11a). However, the slope of the line was greater than 1 indicating that the P concentration in microwave digested samples was higher as compared to that in dry ashed samples (95 % confidence interval for β_1 is 1.05 to 1.13). The best association was observed between perchloric acid digestion and dry ashing (correlation coefficient = 99 %) (Fig 4-11b). In contrast to microwave digest, the

perchloric acid digestion method showed almost 1:1 relationship with dry ashing method (95 % confidence interval for β_1 is 1.00 to 1.10). Though the correlation between microwave digestion and perchloric acid digestion was 0.96, the slope of the regression line indicated that perchloric acid digestion yielded lower concentrations than microwave digestion (95 % confidence interval for β_1 is 0.83 to 0.92).

The association of different digestion techniques for K concentration are illustrated in Fig 4.14. A very high correlation (0.98) was observed for K concentration between microwave digestion and dry ashing with chemical standard calibration (Fig 4-14a). Almost all data points were close to the regression line. However, as evident from the slope of regression line, exact 1:1 relationship does not exist, indicating that the K concentration was higher in microwave digested samples than that of dry ashed samples (95 % confidence interval β_1 is 1.09 to 1.16). Perchloric acid digestion also showed a strong linear association with dry ashing (Fig 4-14b) (correlation coefficient = 0.94 %). But similar to the microwave digestion, the perchloric acid digestion yielded higher concentration than dry ashing, (95 % confidence interval for β_1 is 1.09 to 1.22). Association between microwave digestion and perchloric acid digestion is illustrated in Fig 4-14c. Similar to dry ashing, microwave digestion showed a strong correlation with perchloric acid digestion (correlation coefficient = 0.94). The slope of the line showed a 1:1 relation ship between the two digestion methods (95% confidence interval for β_1 is 0.94 to 1.06).

The scatter plots for calcium concentration obtained from dry ashed, microwave, and perchloric acid digested samples are illustrated in Fig 4-15. Both microwave and

perchloric acid digestion showed a close association with dry ashing. The relationship of dry ashing with microwave digestion was stronger than with perchloric acid digestion (correlation coefficient for microwave digestion and perchloric acid digestion with dry ashing = 0.94 and 0.86 respectively). However, both methods showed slightly higher Ca concentrations than dry ashing (95 % confidence interval for β_1 for microwave digestion and perchloric acid digestion is 1.11 to 1.25 and 0.87 to 1.05 respectively). The association of perchloric acid digestion with microwave digestion (Fig 4-15c) was almost similar to that of dry ashing, (correlation coefficient = 0.86). However, the microwave digestion yielded higher concentrations than the perchloric acid digestion (95 % confidence interval for β_1 is 0.68 to 0.84).

Similar results were observed for Mg (Fig 4-16). The concentration in both microwave digested and perchloric acid digestion sample showed a close relationship with that of dry ashed samples (Fig 4-16a and 4-16b). Also similar to Ca, the microwave digestion showed more strong association with dry ashing (correlation coefficient = 0.95) than perchloric acid digestion (correlation coefficient = 0..85). Microwave digestion yielded higher concentration than dry ashing (95% confidence interval for β_1 is 1.15 to 1.27). On the other hand, the Mg concentration obtained by perchloric acid digestion was not different from that of dry ashing, the slope of the line showed a 1:1 relation ship between the two methods (95 % confidence interval for β_1 is 0.90 to 1.11).

The association of different digestion techniques for S, Mn, B and Cu concentrations is shown in figs 4-17 and 4-18. For S, Mn and B concentration, although the data points showed some trend of association between dry ashing and microwave

digestion, the relation ship was not as strong (correlation coefficient ranged 0.81 to 0.46) as observed for the other elements. Also contrary to the other elements, Cu concentration obtained by microwave digestion, perchloric acid digestion or by dry ashing showed no association among the three digestion methods (Fig 4-18). Generally the data points in almost all cases showed very sparse and random pattern.

In case of Zn, the distribution of the data points followed a linear pattern but with some outliers, in the data obtained from microwave digestion (Fig 4-19) However, the relationship improved considerably when the regression lines were fitted after the outlying values showed by box and whisker plots were excluded from the data. Dry ashing showed good relationship with both microwave and perchloric acid digestion (correlation coefficient = 0.90 and 0.92 for microwave digest and perchloric acid digest, respectively). Similarly, a close association between perchloric acid digestion and microwave digestion was observed, (correlation coefficient = 0.899).

Though the results were statistically significant, the data illustrated that dry ashing performed as well as microwave digestion for the determination of elemental concentration in grass samples, and both digestion methods gave acceptable values for plant nutrition diagnostic purposes for P, K, Ca, Mg. In contrast, the bigger differences in concentration were also observed for S, Mn, Cu, B, and Zn, dry ashing yielded lower concentration than microwave digestion, suggesting that dry ashing caused volatility losses of these nutrients, and these losses were considerably greater for S, Mn and Cu than other elements. Along with volatility losses, dry ashing of tissue samples can also cause precipitation of insoluble silicates and unburned carbon which occlude inorganic

constituents, thereby resulting lower concentration when compared to wet ashing. The advantage of microwave digestion over dry ashing is that microwave digestion is fast, simple and as performed in closed vessels, is less subject to volatility losses. However the speed of microwave digestion procedure becomes less meaningful as the number of samples to be digested increases, since the microwave in our lab can only accommodate only 12 samples. Moreover, a microwave digestion unit costs more than a muffle furnace. Therefore, determination of elemental concentration in plant samples using microwave digestion is more expensive than dry ashing. If the purpose is to perform routine plant analysis involving nutritional diagnosis of major elements in grass samples, dry ashing can successfully be used, since dry ashing procedure is more effective with regard to processing large number of samples. On the other hand, if the analysis is for research purpose or trace level work, which requires high precision and accuracy and if micronutrients are of primary concern, microwave digestion is a more reliable method.

One advantage of microwave digestion over perchloric acid digestion is that radiation energy is applied directly to the sample and excites molecules to boil the solution, thereby providing better control of heating time and power. Moreover, microwave digestion technique requires less operator attention. On the other hand, perchloric acid digestion is performed on a hot plate, where the heat must be first applied to sample container and the transferred to sample, making the process more time and energy consuming. Also, continuous operator attention is needed in perchloric acid digestion to avoid drying of the sample which could cause an explosion hazard. However, these limitations of perchloric acid digestion are somewhat overridden when

the cost of samples analysis is considered, since perchloric acid is a cheaper method than microwave digestion. Since concentrations obtained using either microwave assisted digestion or perchloric acid digestion were comparable for P, K, Ca, and Mg in the grass tissues samples, to choose one method as being the best is difficult and a matter of preference on the basis of analytical limitations and cost, rather than precision. On the other hand, if the major interest is micronutrients such as Cu or Zn it is suggested that microwave digestion is a better choice than perchloric acid digestion.

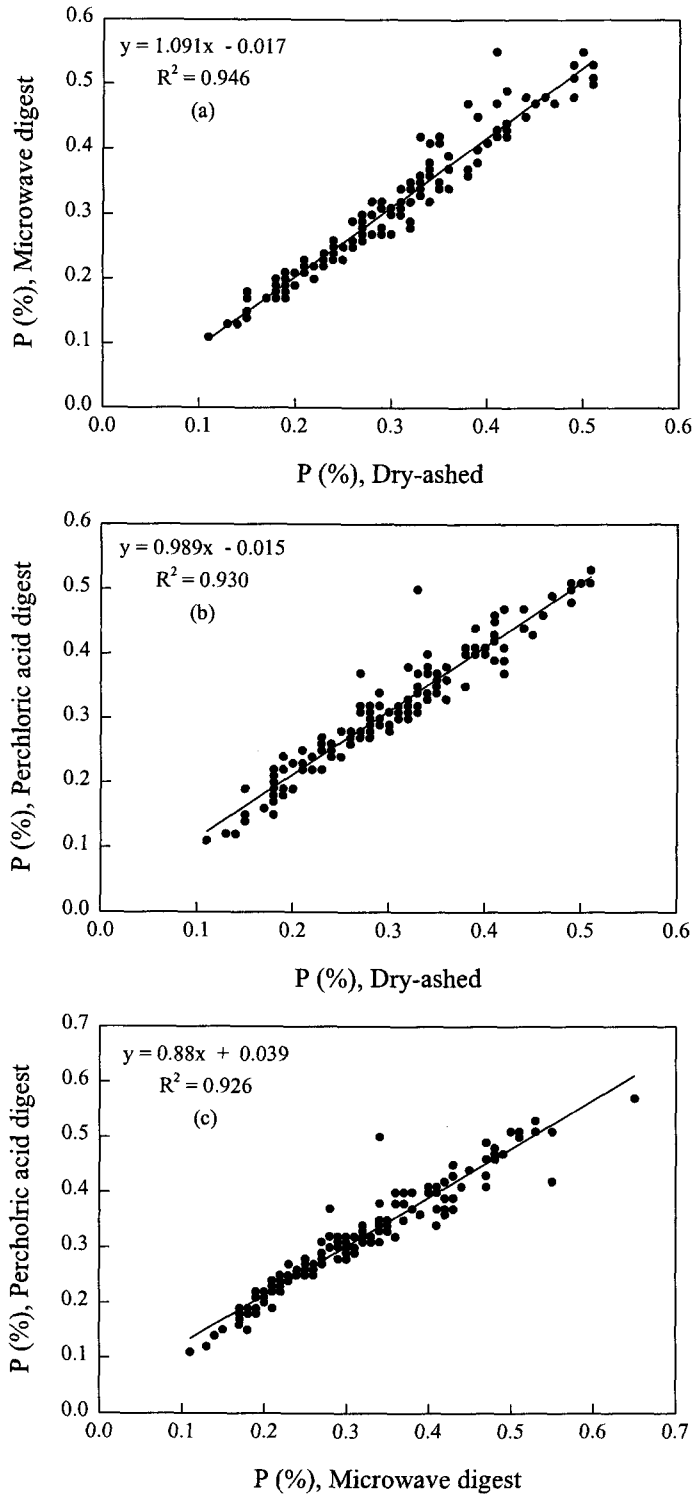

Figure 4-13. Comparisons of dry ashing, microwave digestion and perchloric acid digestion for phosphorus concentration.

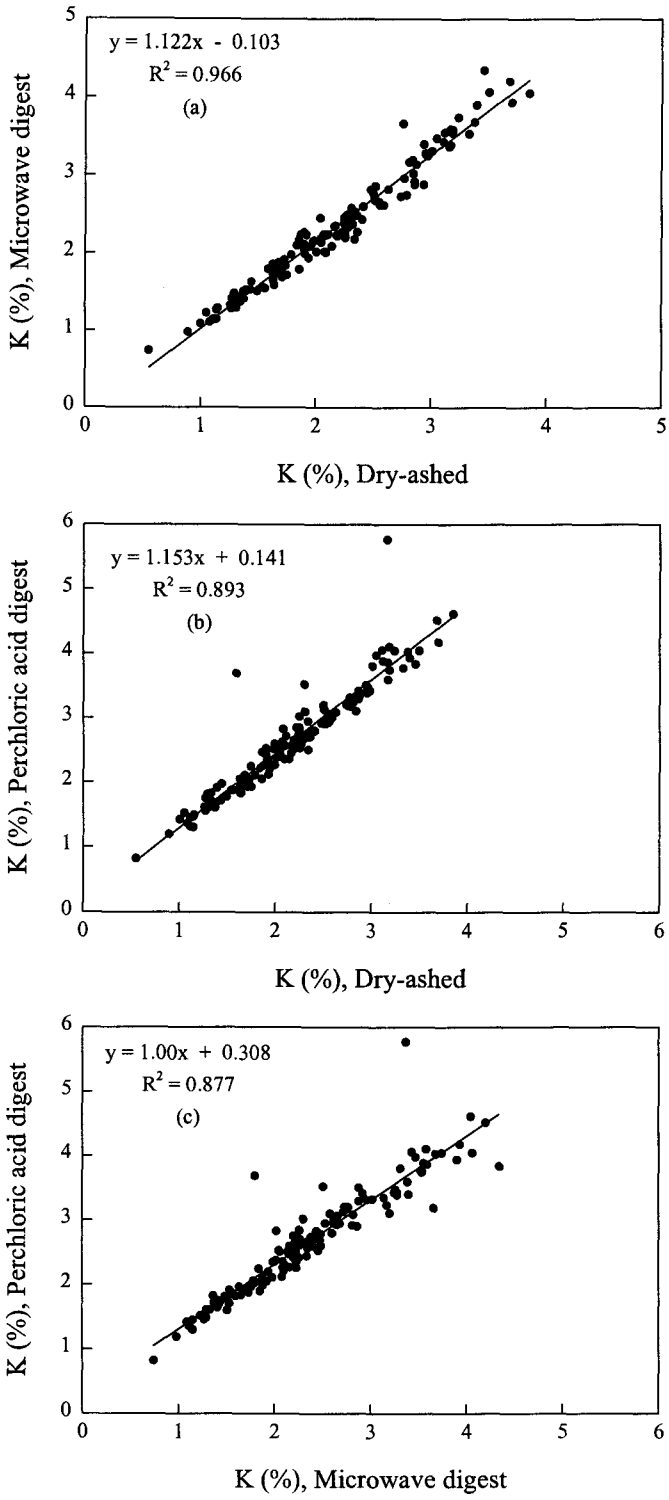

Figure 4-14. Comparisons of dry ashing, microwave digestion and perchloric acid digestion for potassium concentration

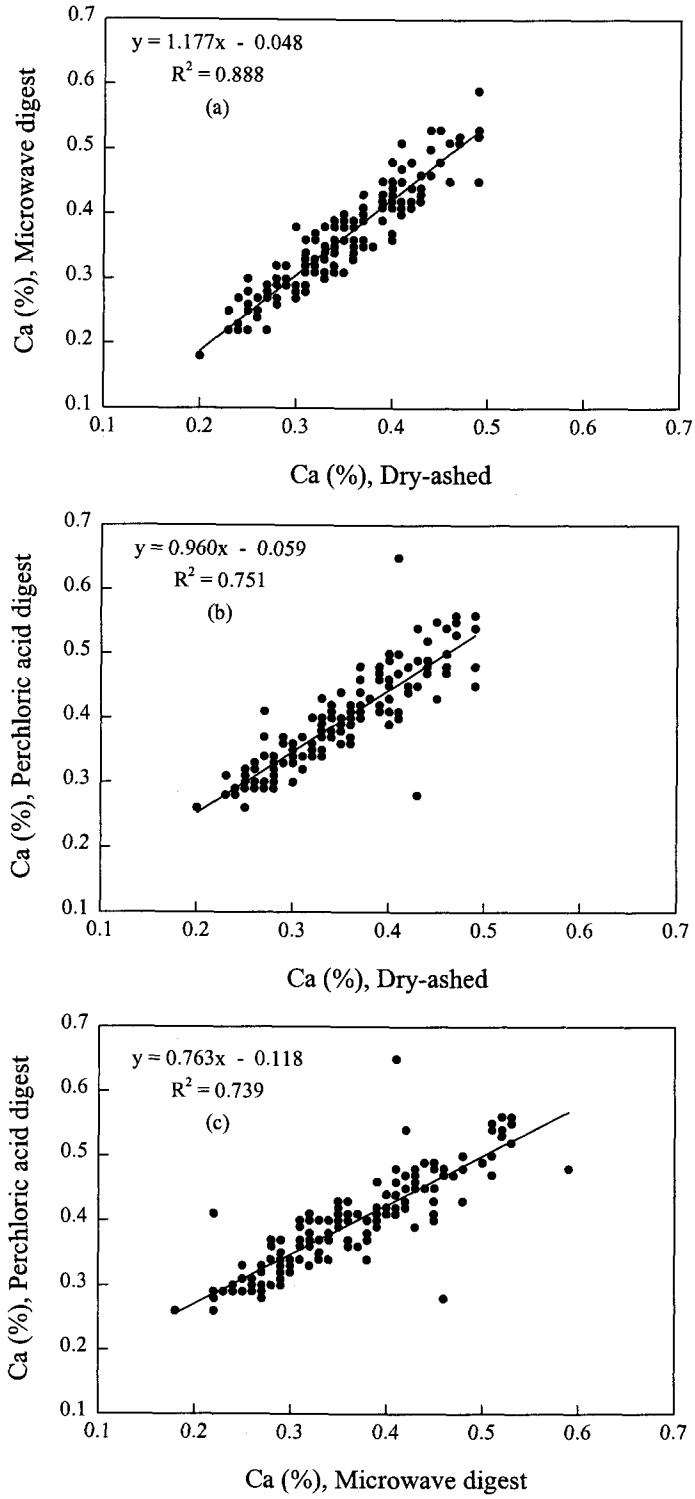

Figure 4-15. Comparisons of dry ashing, microwave digestion and perchloric acid digestion for calcium concentration.

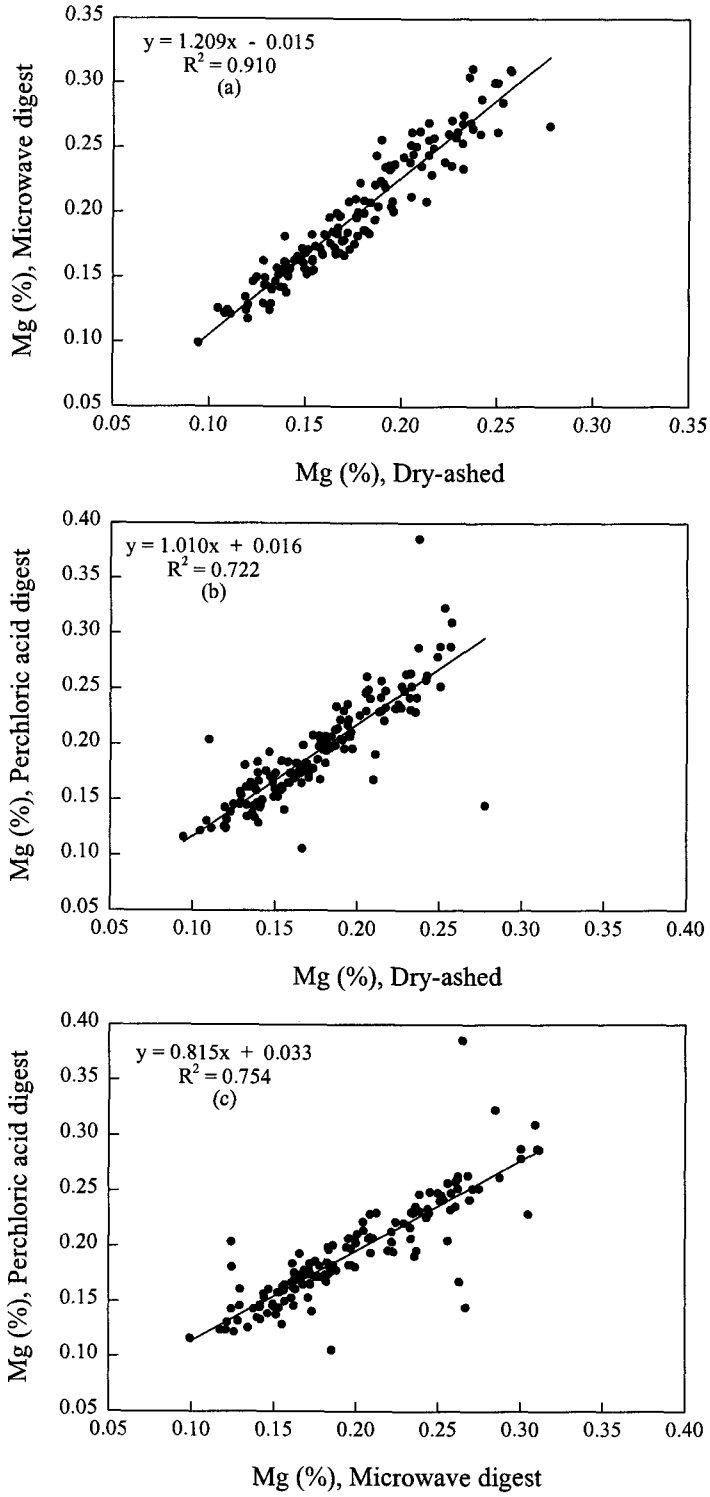

Figure 4-16. Comparisons of dry ashing, microwave digestion and perchloric acid digestion for magnesium concentration.

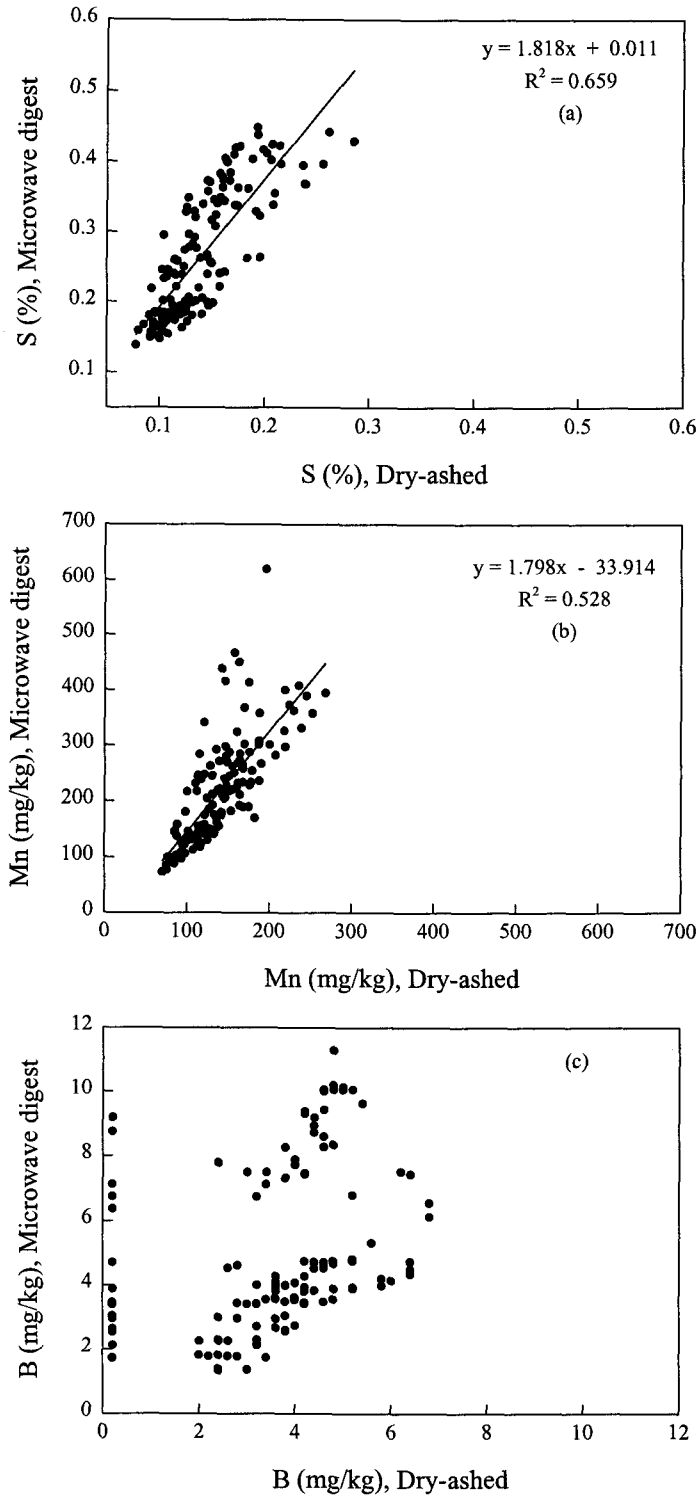

Figure 4-17. Comparisons of dry ashing, microwave digestion for sulfur, manganese and boron concentration.

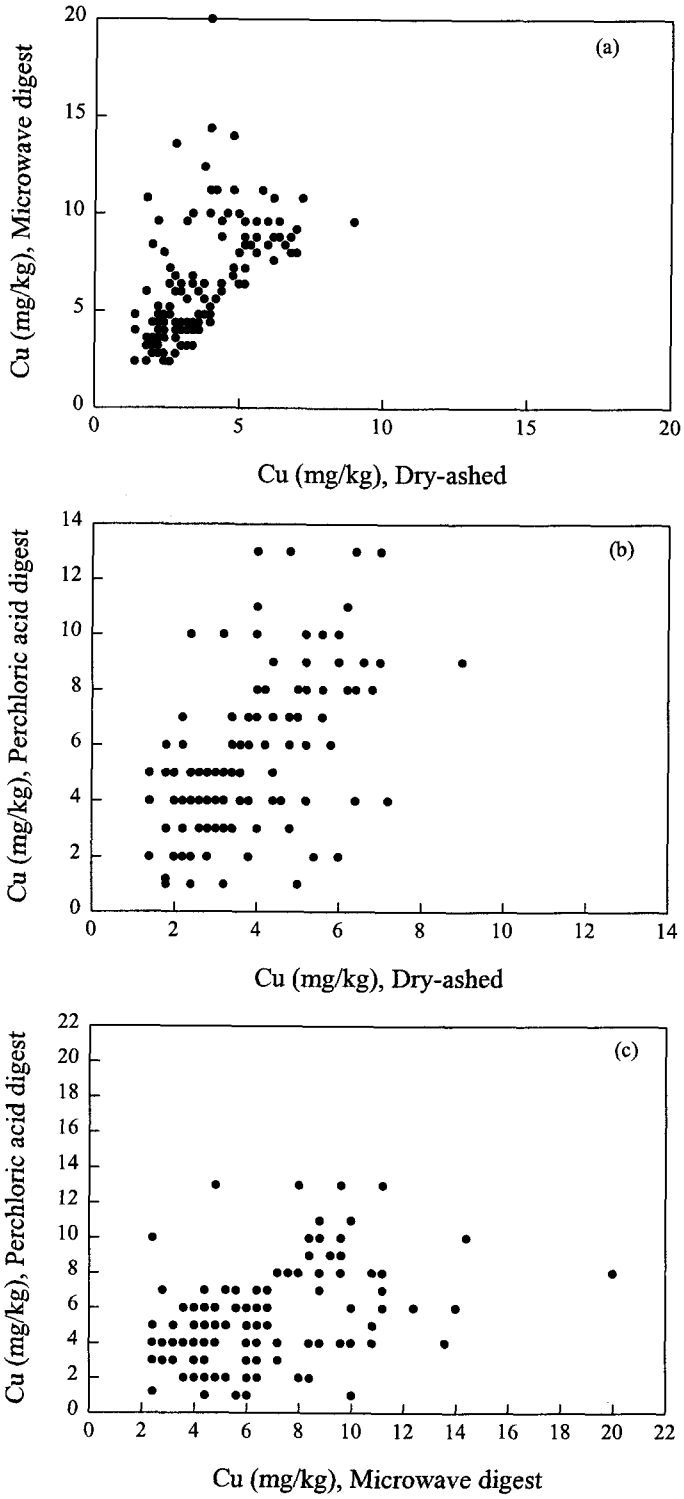

Figure 4-18. Comparisons of dry ashing, microwave digestion and perchloric acid digestion for copper concentration.

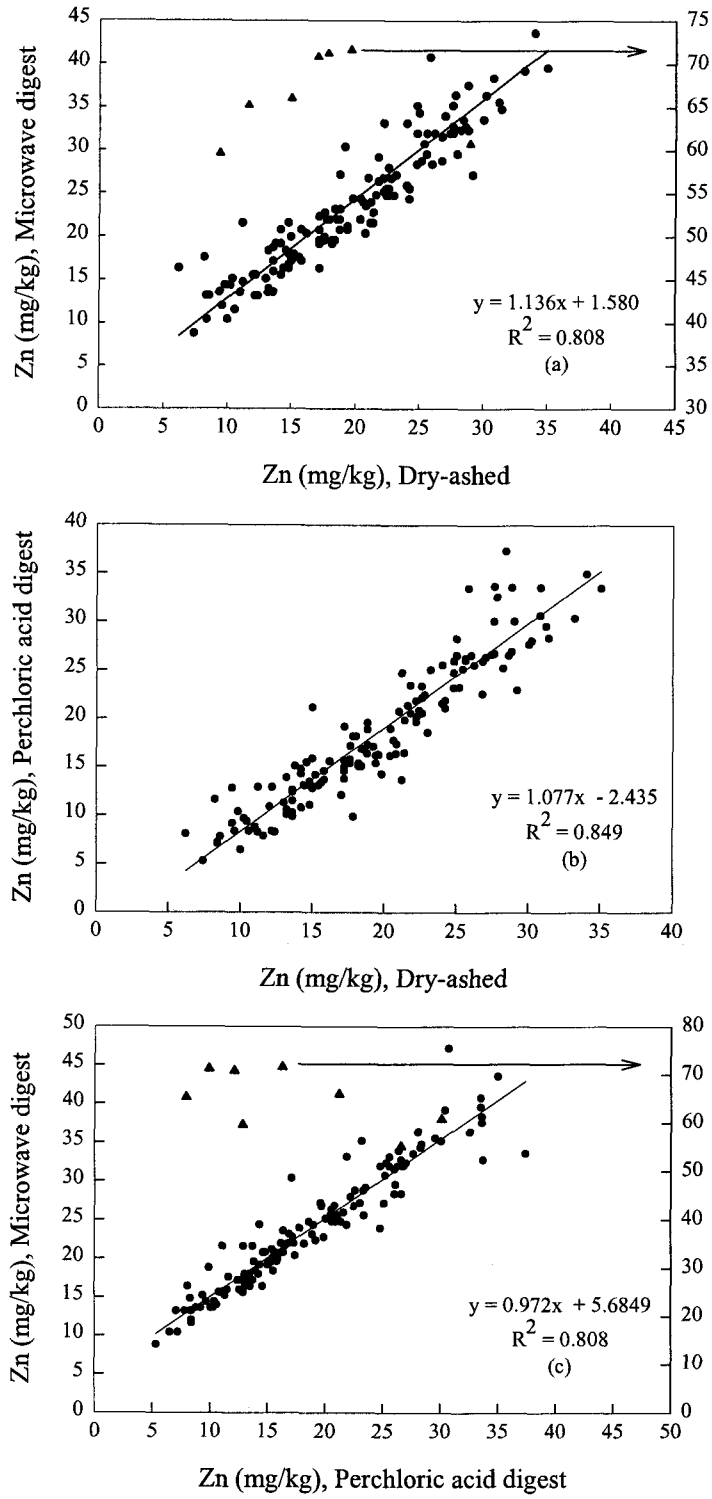

Figure 4-19. Comparisons of dry ashing, microwave digestion and perchloric acid digestion for zinc concentration

CONCLUSIONS

Calibration of the ICP with a dry ashed standard showed no correlation with the chemical standard calibration for S, Ca, Mg, Mn, Cu, and B, and a very weak correlation for P and K. The calibration curve generated by the ICP using a dry ashed standard due to volatility losses of these elements from the standard was steeper as compared to the one generated using a chemical standard, which resulted lower concentration of the samples when the dry ashed standard was used. This suggests, that using a dry ashed standard calibration is not a viable method for the elemental determination of grass samples. Comparisons of a microwave digested standard with a chemical standard calibration showed that both standards yielded comparable concentrations of all elements except Mn and Zn. Though the differences were significant for P, K, S, and Cu, their magnitude was small, suggesting that either standard can be used for the calibration of ICP. Moreover, the SRMs and chemical standards are relatively expensive to use, therefore, for the routine plant analysis, a microwave digested home developed grass tissue standard is almost as dependable as a chemical standard for the calibration of ICP.

Comparisons of dry ashing with microwave digestion showed that dry ashing caused losses, of S, Mn, Cu, B and Zn, whereas concentrations for P, K, Ca and Mg in the dry ashed samples were almost similar to that obtained from microwave digested samples. However, the differences in the two digestion techniques were remarkable only for S and Mn. Thus, if the purpose of analysis is routine plant nutritional diagnosis for fertilizer recommendations of the major elements, dry ashing can be successfully used.

On the other hand, if the analysis is for a trace level work or a nutritional research which requires higher accuracy and precision and particularly the micronutrients are of interest, dry ashing is not a suitable method for the digestion of grass samples.

The results obtained by perchloric acid digestion were almost similar to that of microwave digestion for the major elements, and both digestion methods showed an excellent correlation. Since both digestion methods are equally effective for the elemental determination of grasses, it is difficult to chose one method being the best on the basis of precision. Though perchloric acid digestion is cheaper than microwave assisted digestion, explosion hazards posed by using perchloric acid, and requirement of specialized fumes hoods limit its use. It is suggested that if microwave digestion is available, number of samples is small, and the analytical cost is not a major concern the use of perchloric acid should be avoided. Thus, our findings suggest that the best suited method for the simultaneous determination of elemental composition of grasses is microwave digestion with chemical standard calibration.

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CHAPTER 5

SUMMARY

Growth, nitrogen uptake and nitrogen use efficiency of wheat were higher when wheat followed a legume as compared to when wheat followed oat in all three growing seasons. Nitrogen use efficiency estimated by isotopic dilution was comparable to that estimated using the "by difference method". The similarity in the two methods suggests that N pool substitution was minimal over the experimental period. If NUE is estimated within 50 to 70 days after N application either method can be used. However, if multiple N rates are used NUE can be estimated more precisely by least square method, an expanded by "difference method", using more data. Contribution of legume N to the succeeding wheat crop showed that N fertilizer rates could be reduced by 44 kg N ha^{-1} without yield losses. On the other hand if wheat is grown after oat fall N application as a starter fertilizer is required to alleviate N deficiency that may limit subsequent crop response to applied N.

Comparable gross mineralization rates were estimated by models considering both remineralization and no remineralization. This suggest that remineralization was minimal and MIT was slow. Indicating mineralization of legume N had occurred earlier in the season. In two growing seasons, wheat following oat showed an immobilization of fertilizer N and the N deficiency in wheat following oat was more pronounced in the second year as compared to the first year. The use of mean pool abundance of mineral

pool from plant ^{15}N data to estimate gross transformation rates provided the best approximation in cases where measurement of ^{15}N in the mineral pool was not possible.

Use of dry ashed standard for calibrating the ICP is not a viable method for determining elemental concentration of forages. Microwave digested samples yielded almost similar results as that of chemical standard, suggesting that either standard can be used to calibrate the ICP. Both dry ashing and microwave digestion yielded similar concentrations for P, K, Ca and Mg. However, S, Mn, B and Zn were lost during dry ashing. This suggests that if only macronutrients are being analyzed, dry ashing could be used. Perchloric acid digestion showed good agreement with microwave digestion for all elements, however, the explosion hazards limit the use of this technique. Our study suggest that the best method for simultaneous determination of elemental composition of grass is microwave digestion with chemical standard calibration of ICP.

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APPENDIX

Appendix. 1. Dry matter yield (DMY), % N, % C, N uptake (NUP), and atom % ^{15}N of winter wheat following either clover or oat at two sampling dates in 1995-96 growing season. Rotation: 1 = Wheat following clover; 2 = Wheat following oat.
N-form: 1 = 0-N; 2 = $^{15}NH_4NO_3$; and 3 = $NH_4{}^{15}NO_3$.

Date	Rotation	N form	Rep	DMY kg ha^{-1}	C %	N %	NUP kg ha^{-1}	Atom% ^{15}N
03/16/96	1	1	1	602.85	43.36	2.68	16.16	---
03/16/96	1	1	2	619.04	42.91	2.36	14.61	---
03/16/96	1	1	3	512.65	42.08	2.77	14.20	---
03/16/96	1	1	4	814.85	43.25	3.15	25.67	---
03/16/96	2	1	1	734.68	41.82	2.23	16.38	---
03/16/96	2	1	2	368.49	40.33	2.15	7.92	---
03/16/96	2	1	3	649.11	43.11	2.42	15.71	---
03/16/96	2	1	4	322.24	42.41	2.34	7.54	---
05/04/96	1	1	1	3530.76	42.99	1.51	53.31	0.3856
05/04/96	1	1	2	2667.35	43.56	1.78	47.48	0.3709
05/04/96	1	1	3	1588.07	43.02	1.50	23.82	0.3689
05/04/96	1	1	4	3338.04	43.53	1.59	53.07	0.3698
05/04/96	1	2	1	7693.67	43.66	1.64	126.18	4.9031
05/04/96	1	2	2	6961.31	44.28	1.77	123.22	4.9677
05/04/96	1	2	3	7539.49	43.86	1.83	137.97	4.6656
05/04/96	1	2	4	8387.49	44.00	2.05	171.94	4.5369
05/04/96	1	3	1	8133.09	43.07	1.56	126.88	4.1223
05/04/96	1	3	2	7986.62	43.56	1.71	136.57	4.4637
05/04/96	1	3	3	6036.22	43.85	1.80	108.65	3.4971
05/04/96	1	3	4	7824.73	43.38	1.80	140.85	3.2748
05/04/96	2	1	1	1603.49	43.07	1.81	29.02	0.3699
05/04/96	2	1	2	1433.89	42.71	1.34	19.21	0.3692
05/04/96	2	1	3	2019.78	43.05	1.28	25.85	0.3683
05/04/96	2	1	4	1210.33	43.14	1.41	17.07	0.3746
05/04/96	2	2	1	6398.54	43.37	1.50	95.98	5.1462
05/04/96	2	2	2	5411.78	43.83	1.56	84.42	6.1750
05/04/96	2	2	3	6753.16	43.13	1.05	70.91	5.4608
05/04/96	2	2	4	5072.58	43.97	1.71	86.74	6.1959
05/04/96	2	3	1	6514.18	43.30	0.87	56.67	5.0933
05/04/96	2	3	2	5573.67	43.37	1.44	80.26	5.5270
05/04/96	2	3	3	7739.93	43.73	1.51	116.87	3.7299
05/04/96	2	3	4	5735.56	43.68	1.80	103.24	4.2036

Appendix. 2. Dry matter yield (DMY), % N, % C, N uptake (NUP), and atom % ^{15}N of winter wheat following either clover or oat at three sampling dates in 1996-97 growing season. Rotation: 1 = Wheat following clover; 2 = Wheat following oat.
N-form: 1 = 0-N; 2 = $^{15}NH_4NO_3$; 3 = $NH_4{}^{15}NO_3$, and 4 = Urea.

Date	Rotation	N form	Rep	DMY kg ha^{-1}	C %	N %	NUP kg ha^{-1}	Atom % ^{15}N
02/22/97	1	1	1	969.80	43.3	3.21	31.13	---
02/22/97	1	1	2	972.63	43.6	3.29	32.00	---
02/22/97	1	1	3	1022.87	43.6	3.31	33.86	---
02/22/97	1	1	4	957.47	43.3	3.25	31.12	---
02/22/97	2	1	1	178.10	43.9	3.70	6.59	---
02/22/97	2	1	2	148.42	43.7	3.47	5.15	---
02/22/97	2	1	3	243.90	43.5	3.36	8.19	---
02/22/97	2	1	4	156.52	43.5	3.23	5.06	---
03/22/97	1	1	1	2180.42	44.2	2.48	54.07	---
03/22/97	1	1	2	2347.73	45.0	2.26	53.06	---
03/22/97	1	1	3	1757.65	45.0	2.29	40.25	---
03/22/97	1	1	4	1970.96	44.7	2.19	43.16	---
03/22/97	1	4	1	2601.39	44.6	4.29	111.60	---
03/22/97	1	4	2	2345.16	45.3	4.41	103.42	---
03/22/97	1	4	3	2436.65	45.1	4.20	102.34	---
03/22/97	1	4	4	2690.05	44.8	4.80	129.12	---
03/22/97	2	1	1	487.02	45.3	2.98	14.51	---
03/22/97	2	1	2	253.66	45.5	3.11	7.89	---
03/22/97	2	1	3	409.92	44.1	2.41	9.88	---
03/22/97	2	1	4	433.56	44.5	2.36	10.23	---
03/22/97	2	4	1	675.92	45.7	4.84	32.71	---
03/22/97	2	4	2	886.66	45.9	4.82	42.74	---
03/22/97	2	4	3	802.62	46.3	4.82	38.69	---
03/22/97	2	4	4	758.93	46.0	4.78	36.28	---
05/02/97	1	1	1	3338.81	44.3	1.07	35.73	0.372
05/02/97	1	1	2	3836.04	44.9	1.18	45.27	0.372
05/02/97	1	1	3	4887.56	44.7	1.18	57.67	0.372
05/02/97	1	1	4	4439.66	44.4	0.94	41.73	0.372
05/02/97	2	1	1	1843.24	44.6	1.24	22.86	0.372
05/02/97	2	1	2	1265.06	44.5	1.30	16.45	0.372
05/02/97	2	1	3	1443.14	44.2	1.23	17.75	0.372
05/02/97	2	1	4	1343.69	43.8	1.25	16.80	0.372
05/02/97	1	2	1	8358.20	45.3	1.37	114.51	3.470
05/02/97	1	2	2	8986.49	45.4	1.49	133.90	3.870
05/02/97	1	2	3	10054.19	45.2	1.22	122.66	3.490
05/02/97	1	2	4	10058.05	44.9	1.42	142.82	3.520

Date	Rotation	N form	Rep	DMY kg ha^{-1}	C %	N %	NUP kg ha^{-1}	Atom % ^{15}N
05/02/97	2	2	1	6127.18	45.0	1.08	66.17	3.840
05/02/97	2	2	2	5471.14	44.7	1.08	59.09	4.520
05/02/97	2	2	3	5724.00	45.0	1.40	80.14	4.940
05/02/97	2	2	4	6572.77	44.4	0.98	64.41	4.520
05/02/97	1	3	1	8639.58	45.5	1.44	124.41	3.690
05/02/97	1	3	2	9300.25	45.2	1.66	154.38	3.560
05/02/97	1	3	3	11849.64	45.0	1.44	170.63	3.330
05/02/97	1	3	4	10703.30	44.4	1.46	156.27	3.370
05/02/97	2	3	1	5472.68	45.0	1.59	87.02	3.920
05/02/97	2	3	2	5359.36	45.0	1.32	70.74	4.300
05/02/97	2	3	3	7038.40	44.4	0.75	52.79	4.030
05/02/97	2	3	4	8542.44	44.7	1.23	105.07	4.400

Appendix. 3. Dry matter yield (DMY), % N, % C, N uptake (NUP), and atom % ^{15}N of winter wheat following either clover or oat at three sampling dates in 1997-98 growing season. Rotation: 1 = Wheat following clover; 2 = Wheat following oat.
N-form: 1 = 0-N; 2 = $^{15}NH_4NO_3$; and 3 = $NH_4{}^{15}NO_3$.

Date	Rotation	N form	Rep	DMY kg ha^{-1}	C %	N %	NUP kg ha^{-1}	Atom % ^{15}N
02/18/98	1	1	1	1823.97	39.40	3.65	66.57	0.365
02/18/98	1	1	2	1285.88	38.70	3.16	40.63	0.365
02/18/98	1	1	3	1321.34	41.50	4.33	57.21	0.365
02/18/98	1	1	4	1433.89	43.00	4.02	57.64	0.366
02/18/98	2	1	1	698.44	42.60	3.76	26.26	0.365
02/18/98	2	1	2	595.14	39.70	3.38	20.12	0.365
02/18/98	2	1	3	605.16	40.80	2.79	16.88	0.367
02/18/98	2	1	4	716.17	39.50	3.31	23.71	0.366
03/29/98	1	1	1	2903.50	43.40	2.09	60.68	----
03/29/98	1	1	2	1295.90	43.70	2.31	29.94	----
03/29/98	1	1	3	1525.60	43.60	2.21	33.72	----
03/29/98	1	1	4	524.90	43.90	2.17	11.39	----
03/29/98	2	1	1	623.40	44.40	2.52	15.71	----
03/29/98	2	1	2	672.60	44.00	2.23	15.00	----
03/29/98	2	1	3	1049.90	43.90	2.52	26.46	----
03/29/98	2	1	4	803.80	43.80	2.29	18.41	----
03/26/98	1	2	1	2701.27	44.80	2.86	77.26	2.481
03/29/98	1	2	2	2457.66	44.10	2.70	66.36	2.222
03/29/98	1	2	3	3186.17	44.60	2.30	73.28	1.689
03/29/98	1	2	4	2237.95	44.40	3.01	67.36	2.715
03/29/98	2	2	1	2291.14	44.40	2.81	64.38	2.784
03/29/98	2	2	2	1664.39	44.70	2.39	39.78	2.140
03/29/98	2	2	3	1581.13	44.50	2.77	43.80	2.965
03/29/98	2	2	4	1733.77	44.20	2.89	50.11	3.177
03/29/98	1	3	1	3144.54	44.30	2.62	82.39	1.774
03/29/98	1	3	2	2563.27	43.50	2.58	66.13	1.826
03/29/98	1	3	3	1810.87	44.00	1.97	35.67	1.196
03/29/98	1	3	4	2574.07	44.20	2.82	72.59	2.026
03/29/98	2	3	1	2213.28	44.10	2.60	57.55	2.420
03/29/98	2	3	2	1722.21	44.20	2.43	41.85	2.461
03/29/98	2	3	3	1372.22	44.20	2.32	31.84	2.087
03/29/98	2	3	4	1807.78	44.30	2.67	48.27	2.232
05/01/98	1	1	1	7137.85	39.40	0.96	68.52	----
05/01/98	1	1	2	6839.50	38.70	0.80	54.72	----
05/01/98	1	1	3	6921.28	41.50	0.76	52.60	----
05/01/98	1	1	4	5382.49	43.00	0.92	49.52	----

Date	Rotation	N form	Rep	DMY kg ha^{-1}	C %	N %	NUP kg ha^{-1}	Atom % ^{15}N
05/01/98	2	1	1	4054.21	42.60	0.99	40.14	----
05/01/98	2	1	2	2626.49	39.70	1.03	27.05	----
05/01/98	2	1	3	2504.68	40.80	1.00	25.05	----
05/01/98	2	1	4	2894.76	39.50	0.99	28.66	----
05/01/98	1	2	1	6169.58	44.20	0.90	55.53	1.878
05/01/98	1	2	2	7183.33	44.30	0.97	69.68	2.049
05/01/98	1	2	3	4889.10	44.40	1.08	52.80	1.915
05/01/98	1	2	4	7438.50	44.50	1.25	92.98	2.537
05/01/98	2	2	1	5148.13	44.00	0.94	48.39	2.529
05/01/98	2	2	2	4886.79	44.40	1.07	52.29	3.579
05/01/98	2	2	3	4131.30	44.00	1.02	42.14	1.569
05/01/98	2	2	4	5621.47	44.50	1.06	59.59	3.367
05/01/98	1	3	1	10266.2	44.40	0.92	94.45	1.652
05/01/98	1	3	2	7480.90	44.00	1.16	86.78	1.582
05/01/98	1	3	3	6643.69	44.10	0.80	53.15	1.416
05/01/98	1	3	4	7104.70	44.30	1.20	85.26	1.915
05/01/98	2	3	1	6476.41	44.10	0.76	49.22	2.152
05/01/98	2	3	2	3841.44	44.30	1.07	41.10	2.003
05/01/98	2	3	3	4581.51	43.90	1.21	55.44	1.795
05/01/98	2	3	4	5137.34	44.30	1.13	58.05	2.331

Appendix. 4. Soil ammonium and nitrate-N concentration mg kg^{-1} of soil under winter wheat following either clover or oat at two sampling dates in 1995-96 growing season. Rotation: 1 = Wheat following clover; 2 = Wheat following oat.
N-form: 1 = 0-N; 2 = $^{15}NH_4NO_3$; and 3 = $NH_4{}^{15}NO_3$. Depth: 1= 0 to 10-cm, 2 = 10 to 20-cm.

Date	Rotation	N-form	Rep	Depth	NH_4	NO_3
03/16/96	1	1	1	1	14.6	2.2
03/16/96	1	1	1	2	13.9	2.6
03/16/96	1	1	2	1	40.8	3.4
03/16/96	1	1	2	2	11.6	2.2
03/16/96	1	1	3	1	13.3	3.1
03/16/96	1	1	3	2	8.7	2.5
03/16/96	1	1	4	1	9.0	2.2
03/16/96	1	1	4	2	15.7	1.7
03/16/96	2	1	1	1	14.4	1.9
03/16/96	2	1	1	2	10.0	2.7
03/16/96	2	1	2	1	13.7	1.6
03/16/96	2	1	2	2	9.6	1.7
03/16/96	2	1	3	1	11.6	2.4
03/16/96	2	1	3	2	8.5	2.4
03/16/96	2	1	4	1	12.6	1.4
03/16/96	2	1	4	2	10.9	2.3
05/04/96	1	2	1	1	4.6	0.2
05/04/96	1	2	1	2	5.3	<0.2
05/04/96	1	2	2	1	5.2	0.6
05/04/96	1	2	2	2	4.4	0.4
05/04/96	1	2	3	1	4.4	0.2
05/04/96	1	2	3	2	3.1	0.3
05/04/96	1	2	4	1	4.9	0.4
05/04/96	1	2	4	2	2.5	2.1
05/04/96	1	3	1	1	3.7	<0.2
05/04/96	1	3	1	2	3.7	<0.2
05/04/96	1	3	2	1	4.0	<0.2
05/04/96	1	3	2	2	3.8	<0.2
05/04/96	1	3	3	1	6.0	<0.2
05/04/96	1	3	3	2	3.5	<0.2
05/04/96	1	3	4	1	5.1	0.8
05/04/96	1	3	4	2	4.0	<0.2
05/04/96	2	2	1	1	4.6	0.4
05/04/96	2	2	1	2	3.7	0.2
05/04/96	2	2	2	1	6.5	0.9
05/04/96	2	2	2	2	4.6	0.9

Date	Rotation	N-form	Rep	Depth	NH_4	NO_3
05/04/96	2	2	3	1	5.0	0.2
05/04/96	2	2	3	2	2.8	<0.2
05/04/96	2	2	4	1	3.5	<0.2
05/04/96	2	2	4	2	3.2	0.4
05/04/96	2	3	1	1	3.4	<0.2
05/04/96	2	3	1	2	3.5	<0.2
05/04/96	2	3	2	1	1.6	4.4
05/04/96	2	3	2	2	2.8	0.2
05/04/96	2	3	3	1	6.2	0.7
05/04/96	2	3	3	2	4.6	<0.2
05/04/96	2	3	4	1	4.3	<0.2
05/04/96	2	3	4	2	3.9	<0.2

Appendix. 5. Soil ammonium and nitrate-N concentration mg kg^{-1} of soil under winter wheat following either clover or oat at two sampling dates in 1996-97 growing season. Rotation: 1 = Wheat following clover; 2 = Wheat following oat.
N-form: 1 = 0-N; 2 = $^{15}NH_4NO_3$; and 3 = $NH_4{}^{15}NO_3$. Depth: 1= 0 to 10-cm, 2 = 10 to 20-cm.

Date	Rotation	N-form	Rep	Depth	NH_4	NO_3
02/22/97	1	1	1	1	3.0	1.2
02/22/97	1	1	1	2	2.5	1.0
02/22/97	2	1	1	1	3.1	1.5
02/22/97	2	1	1	2	2.3	1.3
02/22/97	1	1	2	1	3.5	1.1
02/22/97	1	1	2	2	2.4	1.0
02/22/97	2	1	2	1	2.8	1.2
02/22/97	2	1	2	2	2.6	0.9
02/22/97	1	1	3	1	2.6	<.2
02/22/97	1	1	3	2	2.9	1.0
02/22/97	2	1	3	1	2.1	<.2
02/22/97	2	1	3	2	2.1	<.2
02/22/97	2	1	4	1	2.4	<.2
02/22/97	2	1	4	2	2.4	<.2
02/22/97	1	1	4	1	3.0	<.2
02/22/97	1	1	4	2	2.8	<.2
05/22/97	1	1	1	1	4.6	1.4
05/22/97	1	1	2	1	7.0	1.0
05/22/97	1	1	3	1	3.5	1.2
05/22/97	1	1	4	1	3.9	1.2
05/22/97	1	1	1	2	3.8	1.1
05/22/97	1	1	2	2	3.2	0.2
05/22/97	1	1	3	2	3.2	1.1
05/22/97	1	1	4	2	2.8	0.2
05/22/97	1	2	1	1	3.4	0.8
05/22/97	1	2	2	1	3.9	0.8
05/22/97	1	2	3	1	3.5	1.3
05/22/97	1	2	4	1	3.9	0.9
05/22/97	1	2	1	2	3.1	0.2
05/22/97	1	2	2	2	3.4	0.9
05/22/97	1	2	3	2	3.1	1.1
05/22/97	1	2	4	2	3.6	0.2
05/22/97	1	3	1	1	4.4	0.9
05/22/97	1	3	2	1	4.4	0.8
05/22/97	1	3	3	1	3.6	0.2
05/22/97	1	3	4	1	4.2	0.2

Date	Rotation	N-form	Rep	Depth	NH_4	NO_3
05/22/97	1	3	1	2	3.7	1.0
05/22/97	1	3	2	2	3.3	0.2
05/22/97	1	3	3	2	3.4	0.8
05/22/97	1	3	4	2	3.1	0.2
05/22/97	2	1	1	1	3.5	1.2
05/22/97	2	1	2	1	3.9	1.1
05/22/97	2	1	3	1	3.2	0.8
05/22/97	2	1	4	1	3.4	0.8
05/22/97	2	1	1	2	3.8	0.9
05/22/97	2	1	2	2	3.3	1.2
05/22/97	2	1	3	2	3.5	0.2
05/22/97	2	1	4	2	3.7	0.2
05/22/97	2	2	1	1	4.6	1.1
05/22/97	2	2	2	1	3.6	1.0
05/22/97	2	2	3	1	3.6	0.8
05/22/97	2	2	4	1	3.2	0.2
05/22/97	2	2	1	2	3.7	0.9
05/22/97	2	2	2	2	3.5	0.2
05/22/97	2	2	3	2	3.1	0.2
05/22/97	2	2	4	2	3.4	0.2
05/22/97	2	3	1	1	3.9	0.9
05/22/97	2	3	2	1	3.9	1.0
05/22/97	2	3	3	1	3.3	0.8
05/22/97	2	3	4	1	3.2	0.2
05/22/97	2	3	1	2	3.3	0.8
05/22/97	2	3	2	2	3.4	1.2
05/22/97	2	3	3	2	3.3	0.9
05/22/97	2	3	4	2	2.9	0.2

Appendix. 6. Soil ammonium and nitrate-N concentration mg kg^{-1} of soil under winter wheat following either clover or oat at three sampling dates in 1997-98 growing season. Rotation: 1 = Wheat following clover; 2 = Wheat following oat.
N-form: 1 = 0-N; 2 = $^{15}NH_4NO_3$; and 3 = $NH_4{}^{15}NO_3$. Depth: 1= 0 to 10-cm, 2 = 10 to 20-cm.

Date	Rotation	N-form	Depth	Rep	NH_4	NO_3
02/18/98	1	1	1	1	4.45	0.70
02/18/98	1	1	1	2	4.60	0.75
02/18/98	1	1	1	3	3.55	1.50
02/18/98	1	1	1	4	3.65	1.50
02/18/98	1	1	2	1	2.90	0.80
02/18/98	1	1	2	2	4.45	0.50
02/18/98	1	1	2	3	---	---
02/18/98	1	1	2	4	---	---
02/18/98	2	1	1	1	4.50	2.35
02/18/98	2	1	1	2	3.50	0.35
02/18/98	2	1	1	3	3.30	1.50
02/18/98	2	1	1	4	3.40	1.50
02/18/98	2	1	2	1	3.45	0.35
02/18/98	2	1	2	2	3.00	0.35
02/18/98	2	1	2	3	---	---
02/18/98	2	1	2	4	---	---
03/26/98	1	2	1	1	3.50	1.50
03/26/98	1	2	1	2	4.50	1.50
03/26/98	1	2	1	3	3.40	1.50
03/26/98	1	2	1	4	3.90	1.50
03/26/98	1	2	2	1	2.60	1.50
03/26/98	1	2	2	2	3.30	1.50
03/26/98	1	2	2	3	3.00	1.50
03/26/98	1	2	2	4	2.80	1.50
03/26/98	1	3	1	1	4.70	1.50
03/26/98	1	3	1	2	3.80	1.50
03/26/98	1	3	1	3	3.70	1.50
03/26/98	1	3	1	4	4.00	1.50
03/26/98	1	3	2	1	3.00	1.50
03/26/98	1	3	2	2	2.80	1.50
03/26/98	1	3	2	3	3.30	1.50
03/26/98	1	3	2	4	2.60	1.50
03/26/98	2	2	1	1	4.80	1.50
03/26/98	2	2	1	2	3.80	1.50
03/26/98	2	2	1	3	4.60	1.50
03/26/98	2	2	1	4	4.40	1.50

Date	Rotation	N-form	Depth	Rep	NH_4	NO_3
03/26/98	2	2	2	1	5.70	1.50
03/26/98	2	2	2	2	4.70	1.50
03/26/98	2	2	2	3	3.10	1.50
03/26/98	2	2	2	4	3.10	1.50
03/26/98	2	3	1	1	3.20	1.50
03/26/98	2	3	1	2	3.90	1.50
03/26/98	2	3	1	3	5.00	1.50
03/26/98	2	3	1	4	3.50	1.50
03/26/98	2	3	2	1	4.00	1.50
03/26/98	2	3	2	2	2.80	1.50
03/26/98	2	3	2	3	3.60	1.50
03/26/98	2	3	2	4	4.30	1.50
05/01/98	1	1	1	1	---	---
05/01/98	1	1	1	2	---	---
05/01/98	1	1	1	3	---	---
05/01/98	1	1	1	4	---	---
05/01/98	1	1	2	1	---	---
05/01/98	1	1	2	2	---	---
05/01/98	1	1	2	3	---	---
05/01/98	1	1	2	4	---	---
05/01/98	1	2	1	1	4.40	<1.5
05/01/98	1	2	1	2	4.20	<1.5
05/01/98	1	2	1	3	4.30	<1.5
05/01/98	1	2	1	4	3.70	<1.5
05/01/98	1	2	2	1	4.00	<1.5
05/01/98	1	2	2	2	2.80	<1.5
05/01/98	1	2	2	3	5.60	<1.5
05/01/98	1	2	2	4	2.90	<1.5
05/01/98	1	3	1	1	3.90	<1.5
05/01/98	1	3	1	2	4.00	<1.5
05/01/98	1	3	1	3	6.60	<1.5
05/01/98	1	3	1	4	4.00	<1.5
05/01/98	1	3	2	1	4.70	<1.5
05/01/98	1	3	2	2	5.10	<1.5
05/01/98	1	3	2	3	2.80	<1.5
05/01/98	1	3	2	4	2.80	<1.5
05/01/98	2	1	1	1	---	---
05/01/98	2	1	1	2	---	---
05/01/98	2	1	1	3	---	---
05/01/98	2	1	1	4	---	---
05/01/98	2	1	2	1	---	---
05/01/98	2	1	2	2	---	---

Date	Rotation	N-form	Depth	Rep	NH_4	NO_3
05/01/98	2	1	2	3	---	---
05/01/98	2	1	2	4	---	---
05/01/98	2	2	1	1	5.30	<1.5
05/01/98	2	2	1	2	6.10	<1.5
05/01/98	2	2	1	3	4.60	<1.5
05/01/98	2	2	1	4	3.80	<1.5
05/01/98	2	2	2	1	5.00	<1.5
05/01/98	2	2	2	2	3.20	<1.5
05/01/98	2	2	2	3	2.90	<1.5
05/01/98	2	2	2	4	2.70	<1.5
05/01/98	2	3	1	1	3.70	<1.5
05/01/98	2	3	1	2	3.80	<1.5
05/01/98	2	3	1	3	3.60	<1.5
05/01/98	2	3	1	4	4.10	<1.5
05/01/98	2	3	2	1	3.80	<1.5
05/01/98	2	3	2	2	5.90	<1.5
05/01/98	2	3	2	3	3.50	<1.5
05/01/98	2	3	2	4	2.80	<1.5

Appendix. 7. Soil organic + inorganic N (%), C (%) and atom % ^{15}N under winter wheat following either clover or oat in three growing seasons.
Rotation: 1 = Wheat following clover; 2 = Wheat following oat.
Year: 1 = 1995-96; 2 = 1996-97 and 3 = 1997-98. N-form: 1 = $^{15}NH_4NO_3$; and 2 = $NH_4{}^{15}NO_3$. Depth: 1= 0 to 10-cm, 2 = 10 to 20-cm.

Year	Rotation	N-form	Depth	N	C	Atom % ^{15}N
1	1	1	1	0.12	1.60	0.3918
1	1	1	2	0.11	1.43	0.3882
1	1	2	1	0.12	1.56	0.4057
1	1	2	2	0.11	1.48	0.3766
1	2	1	1	0.11	1.52	0.3987
1	2	1	2	0.11	1.40	0.4088
1	2	2	1	0.12	1.57	0.3797
1	2	2	2	0.11	1.51	0.3805
2	1	1	1	0.11	1.53	0.4082
2	1	1	2	0.11	1.52	0.3886
2	1	2	1	0.11	1.56	0.3963
2	1	2	2	0.11	1.49	0.3793
2	2	1	1	0.11	1.48	0.4148
2	2	1	2	0.11	1.46	0.4024
2	2	2	1	0.11	1.52	0.3889
2	2	2	2	0.11	1.47	0.3761
3	1	1	1	0.12	1.68	0.3893
3	1	1	2	0.11	1.49	0.3778
3	1	2	1	0.12	1.69	0.3851
3	1	2	2	0.10	1.41	0.3697
3	2	1	1	0.11	1.54	0.4197
3	2	1	2	0.11	1.44	0.3768
3	2	2	1	0.11	1.58	0.3764
3	2	2	2	0.11	1.48	0.3727

Appendix. 8. Phosphorus concentration (%) of Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard; V = Perchloric acid digest sample/Chemical standard.

Method Sample #	**I**	**II**	**III**	**IV**	**V**
101	0.580	0.546	0.646	0.640	0.571
102	0.410	0.463	0.479	0.470	0.458
103	0.460	0.487	0.527	0.520	0.510
104	0.460	0.510	0.527	0.530	0.533
105	0.460	0.511	0.511	0.520	0.512
106	0.450	0.493	0.508	0.540	0.495
110	0.380	0.420	0.416	0.420	0.393
111	0.220	0.353	0.341	0.340	0.339
112	0.310	0.386	0.383	0.380	0.405
113	0.340	0.387	0.403	0.420	0.396
114	0.340	0.358	0.343	0.340	0.325
115	0.340	0.378	0.373	0.360	0.345
119	0.210	0.310	0.302	0.300	0.309
120	0.270	0.300	0.303	0.290	0.280
121	0.250	0.328	0.335	0.340	0.346
122	0.190	0.319	0.323	0.330	0.335
123	0.200	0.331	0.341	0.330	0.342
124	0.200	0.322	0.337	0.340	0.326
128	0.260	0.399	0.410	0.410	0.396
129	0.350	0.422	0.431	0.420	0.369
130	0.340	0.417	0.440	0.430	0.406
131	0.260	0.444	0.454	0.450	0.437
132	0.350	0.453	0.469	0.470	0.435
133	0.220	0.401	0.406	0.410	0.409
201	0.210	0.497	0.546	0.540	0.507
202	0.230	0.509	0.504	0.510	0.513
203	0.300	0.472	0.472	0.470	0.491
204	0.170	0.485	0.478	0.490	0.484
205	0.200	0.442	0.476	0.500	0.473
206	0.260	0.408	0.425	0.440	0.416
207	0.310	0.333	0.349	0.370	0.340
208	0.250	0.312	0.307	0.310	0.302
209	0.290	0.321	0.348	0.360	0.328
210	0.220	0.314	0.317	0.320	0.310
211	0.220	0.291	0.311	0.310	0.300

Method Sample #	I	II	III	IV	V
212	0.140	0.331	0.326	0.330	0.317
213	0.200	0.280	0.298	0.310	0.299
214	0.220	0.328	0.329	0.330	0.317
215	0.200	0.298	0.308	0.290	0.287
216	0.240	0.329	0.342	0.330	0.502
217	0.190	0.310	0.341	0.330	0.312
218	0.200	0.329	0.356	0.330	0.324
219	0.260	0.409	0.430	0.410	0.393
220	0.350	0.327	0.416	0.400	0.369
221	0.320	0.340	0.413	0.390	0.335
222	0.290	0.344	0.356	0.360	0.378
223	0.270	0.352	0.417	0.410	0.363
224	0.260	0.380	0.467	0.450	0.406
301	0.410	0.406	0.428	0.450	0.450
302	0.320	0.425	0.492	0.540	0.469
303	0.290	0.394	0.454	0.500	0.436
304	0.400	0.405	0.434	0.460	0.429
305	0.360	0.407	0.471	0.510	0.457
306	0.190	0.276	0.324	0.350	0.322
307	0.280	0.285	0.314	0.350	0.315
308	0.190	0.279	0.304	0.350	0.302
309	0.210	0.339	0.382	0.410	0.371
310	0.190	0.285	0.299	0.300	0.294
311	0.250	0.276	0.319	0.320	0.309
312	0.180	0.313	0.299	0.310	0.321
314	0.230	0.271	0.297	0.310	0.281
315	0.180	0.257	0.293	0.290	0.281
317	0.250	0.288	0.317	0.290	0.342
318	0.210	0.280	0.302	0.250	0.292
319	0.250	0.341	0.367	0.360	0.400
320	0.270	0.346	0.413	0.340	0.374
321	0.300	0.387	0.399	0.400	0.406
322	0.220	0.322	0.344	0.340	0.379
323	0.260	0.386	0.382	0.400	0.401
324	0.330	0.413	0.548	0.540	0.425
401	0.270	0.365	0.368	0.370	0.377
402	0.280	0.383	0.361	0.360	0.395
403	0.220	0.333	0.329	0.330	0.309
404	0.260	0.347	0.352	0.350	0.350
405	0.270	0.359	0.386	0.370	0.361
406	0.210	0.337	0.320	0.320	0.331

Method Sample #	I	II	III	IV	V
407	0.210	0.282	0.265	0.260	0.284
408	0.190	0.277	0.269	0.270	0.282
409	0.230	0.279	0.269	0.270	0.278
410	0.200	0.285	0.270	0.270	0.271
411	0.180	0.251	0.233	0.240	0.240
412	0.190	0.259	0.260	0.270	0.257
413	0.170	0.245	0.246	0.240	0.247
414	0.200	0.270	0.268	0.260	0.269
415	0.230	0.245	0.255	0.250	0.247
416	0.210	0.269	0.266	0.270	0.269
417	0.150	0.265	0.246	0.260	0.272
418	0.200	0.271	0.256	0.260	0.266
419	0.190	0.292	0.273	0.280	0.291
420	0.250	0.321	0.292	0.300	0.300
421	0.230	0.324	0.292	0.310	0.314
422	0.240	0.303	0.271	0.280	0.314
423	0.210	0.295	0.282	0.300	0.303
424	0.250	0.321	0.276	0.290	0.319
501	0.200	0.274	0.293	0.310	0.322
502	0.200	0.272	0.269	0.300	0.310
503	0.220	0.254	0.252	0.270	0.278
504	0.180	0.225	0.224	0.240	0.255
505	0.220	0.270	0.279	0.310	0.372
506	0.170	0.236	0.237	0.260	0.264
507	0.160	0.187	0.205	0.220	0.186
508	0.160	0.180	0.197	0.210	0.195
509	0.130	0.194	0.186	0.220	0.218
510	0.150	0.179	0.192	0.210	0.212
511	0.120	0.154	0.166	0.180	0.194
512	0.150	0.192	0.208	0.230	0.238
513	0.160	0.179	0.202	0.210	0.220
514	0.150	0.176	0.202	0.200	0.215
515	0.150	0.186	0.202	0.200	0.217
516	0.160	0.201	0.214	0.210	0.234
517	0.190	0.214	0.230	0.230	0.254
518	0.120	0.154	0.175	0.170	0.185
519	0.180	0.220	0.221	0.220	0.243
520	0.140	0.188	0.201	0.200	0.221
521	0.170	0.241	0.245	0.250	0.253
522	0.170	0.242	0.251	0.250	0.261
523	0.160	0.241	0.239	0.240	0.265

Method Sample #	I	II	III	IV	V
524	0.150	0.231	0.231	0.260	0.274
601	0.170	0.232	0.227	0.230	0.250
602	0.180	0.231	0.235	0.240	0.256
603	0.150	0.206	0.217	0.220	0.230
604	0.160	0.218	0.202	0.200	0.221
605	0.160	0.232	0.229	0.230	0.249
606	0.140	0.207	0.207	0.210	0.224
610	0.110	0.178	0.174	0.170	0.186
611	0.110	0.182	0.176	0.180	0.155
612	0.110	0.146	0.137	0.140	0.138
613	0.120	0.151	0.151	0.150	0.145
614	0.070	0.106	0.107	0.110	0.111
615	0.110	0.187	0.193	0.190	0.181
616	0.140	0.169	0.169	0.160	0.161
617	0.090	0.137	0.130	0.120	0.125
618	0.130	0.125	0.128	0.120	0.116
619	0.200	0.195	0.186	0.200	0.194
620	0.160	0.185	0.172	0.180	0.182
621	0.170	0.184	0.168	0.170	0.168
625	0.180	0.177	0.166	0.170	0.179
626	0.120	0.193	0.172	0.180	0.180
627	0.160	0.242	0.233	0.240	0.235
628	0.200	0.226	0.217	0.220	0.222
629	0.170	0.203	0.186	0.180	0.194
630	0.160	0.194	0.178	0.170	0.183

Appendix. 9. Potassium concentration (%) of Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard; V = Perchloric acid digest sample/Chemical standard.

Method Sample #	I	II	III	IV	V
101	3.30	3.46	4.34	3.90	3.84
102	2.30	2.96	3.26	2.95	3.48
103	2.28	3.05	3.47	3.13	3.98
104	2.51	3.19	3.58	3.25	4.11
105	2.33	3.11	3.43	3.08	4.06
106	2.34	3.12	3.55	3.26	3.89
110	1.87	2.26	2.41	2.19	2.60
111	1.18	2.14	2.09	1.94	2.36
112	1.52	2.22	2.26	2.12	2.85
113	2.05	2.50	2.77	2.60	3.20
114	1.90	2.23	2.27	2.13	2.62
115	1.88	2.36	2.48	2.28	2.79
119	1.27	2.25	2.26	2.13	2.85
120	1.59	2.10	2.23	2.06	2.57
121	1.39	2.17	2.35	2.20	2.57
122	1.51	2.85	3.02	2.85	3.33
123	1.26	2.63	2.82	2.57	3.09
124	1.53	2.84	3.20	2.93	3.11
128	1.17	1.91	2.11	1.94	2.31
129	1.77	2.25	2.46	2.25	2.53
130	2.02	2.52	2.86	2.59	2.91
131	1.66	2.77	2.96	2.79	3.32
132	2.11	2.81	3.17	2.93	3.24
133	1.31	2.51	2.72	2.61	3.13
201	1.54	3.68	4.20	3.92	4.52
202	1.84	3.85	4.05	3.86	4.62
203	2.06	3.17	3.59	3.29	3.87
204	1.51	3.70	3.93	3.66	4.18
205	1.55	3.24	3.74	3.62	4.05
206	1.92	3.19	3.54	3.41	3.75
207	1.45	1.63	1.85	1.76	1.90
208	1.53	1.96	2.09	1.97	2.27
209	1.54	1.90	2.26	2.15	2.43
210	1.33	2.05	2.20	2.06	2.42
211	1.41	1.86	2.16	2.08	2.47

Method Sample #	I	II	III	IV	V
212	0.97	2.16	2.34	2.19	2.45
213	1.20	2.04	2.45	2.29	2.57
214	1.38	2.27	2.49	2.35	2.61
215	1.44	2.41	2.60	2.42	2.80
216	2.26	3.16	3.37	3.17	5.77
217	1.64	2.95	3.28	3.07	3.39
218	1.81	3.17	3.39	3.12	3.60
219	1.47	2.30	2.48	2.33	2.69
220	1.93	1.92	2.23	2.09	2.35
221	2.21	2.48	2.81	2.60	2.92
222	1.99	2.56	2.62	2.41	2.95
223	1.81	2.51	2.68	2.57	2.95
224	1.99	2.97	3.25	3.08	3.43
301	2.87	3.38	3.68	3.39	4.03
302	2.28	3.50	4.06	3.76	4.05
303	1.70	2.94	3.40	3.22	3.40
304	3.03	3.33	3.53	3.34	3.78
305	2.75	3.40	3.90	3.66	3.94
306	1.09	1.68	1.88	1.79	2.11
307	1.51	1.79	1.98	1.88	2.11
308	1.06	1.72	1.88	1.81	1.99
309	1.10	1.99	2.16	2.02	2.27
310	1.08	1.93	2.08	1.92	2.12
311	1.35	1.84	2.10	1.93	2.22
312	1.03	2.34	2.18	2.04	2.51
314	1.13	1.73	1.92	1.78	2.05
315	0.99	1.63	1.86	1.73	2.05
317	2.35	3.01	3.31	3.09	3.81
318	1.84	2.98	3.28	3.02	3.43
319	1.13	1.74	1.84	1.69	2.25
320	1.32	1.87	2.23	2.08	2.26
321	1.70	2.31	2.44	2.30	2.72
322	1.52	2.34	2.53	2.39	2.95
323	1.85	2.74	2.73	2.69	3.21
324	2.16	2.76	3.66	3.53	3.19
401	2.10	2.86	2.92	2.77	3.42
402	2.05	2.94	2.88	2.77	3.51
403	1.49	2.55	2.65	2.52	2.93
404	1.94	2.86	2.88	2.76	3.30
405	1.83	2.87	3.14	2.96	3.35
406	1.46	2.79	2.75	2.62	3.18

Method Sample #	I	II	III	IV	V
407	1.07	1.56	1.55	1.44	1.87
408	0.96	1.49	1.51	1.40	1.77
409	1.35	1.63	1.67	1.56	1.93
410	1.21	1.72	1.72	1.61	1.94
411	1.24	1.71	1.69	1.58	1.93
412	1.19	1.63	1.73	1.64	1.88
413	0.86	1.43	1.53	1.42	1.71
414	1.01	1.70	1.72	1.63	1.94
415	1.01	1.37	1.51	1.40	1.60
416	1.77	2.59	2.62	2.45	3.01
417	1.22	2.40	2.43	2.27	2.81
418	1.49	2.32	2.37	2.22	2.70
419	0.88	1.37	1.41	1.32	1.65
420	1.37	1.75	1.72	1.60	1.93
421	1.54	2.01	2.02	1.89	2.38
422	1.62	2.09	2.01	1.89	2.36
423	1.58	2.18	2.23	2.08	2.50
424	1.81	2.36	2.27	2.11	2.71
501	1.41	2.25	2.44	2.33	2.83
502	1.34	2.11	2.24	2.12	2.72
503	1.48	2.05	2.14	1.97	2.51
504	1.48	2.25	2.30	2.18	3.02
505	1.58	2.30	2.51	2.37	3.52
506	1.12	2.24	2.38	2.20	2.74
507	0.83	1.15	1.29	1.21	1.49
508	0.78	1.14	1.27	1.19	1.46
509	0.77	1.33	1.36	1.35	1.83
510	0.84	1.27	1.41	1.37	1.75
511	0.95	1.39	1.53	1.49	1.91
512	0.86	1.44	1.63	1.57	1.97
513	0.59	1.05	1.23	1.19	1.52
514	0.70	1.29	1.48	1.42	1.81
515	0.84	1.33	1.43	1.35	1.71
516	1.21	1.91	2.06	2.01	2.51
517	1.83	2.31	2.58	2.48	3.10
518	0.88	1.59	1.80	1.72	3.69
519	0.89	1.35	1.38	1.30	1.66
520	0.53	0.89	0.98	0.93	1.19
521	1.15	1.70	1.79	1.74	2.02
522	1.38	2.07	2.24	2.19	2.63
523	0.97	1.63	1.65	1.53	1.84

Method Sample #	I	II	III	IV	V
524	1.25	2.08	2.02	2.19	2.83
601	1.14	1.91	1.99	1.84	2.35
602	1.09	1.98	2.15	2.01	2.52
603	1.13	1.90	2.05	1.92	2.53
604	1.65	2.26	2.20	2.00	2.76
605	1.48	2.55	2.66	2.50	3.07
606	1.01	1.99	2.16	2.00	2.60
610	0.54	1.30	1.37	1.28	1.72
611	0.62	1.26	1.33	1.23	1.61
612	0.84	1.31	1.29	1.19	1.61
613	0.65	1.08	1.11	1.02	1.36
614	0.67	1.27	1.28	1.21	1.55
615	0.72	1.67	1.78	1.65	2.06
616	0.64	1.11	1.14	1.05	1.31
617	0.51	1.14	1.15	1.07	1.30
618	0.40	0.55	0.74	0.70	0.82
619	1.51	1.68	1.76	1.63	1.97
620	1.64	2.28	2.33	2.15	2.66
621	1.46	1.94	1.94	1.79	2.20
625	0.92	1.11	1.15	1.07	1.45
626	0.56	1.00	1.09	1.01	1.42
627	0.88	1.54	1.56	1.44	1.87
628	1.85	2.19	2.22	2.06	2.66
629	1.26	1.64	1.59	1.50	1.83
630	1.36	1.86	1.79	1.70	2.05

Appendix. 10. Sulfur concentration (%) of Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard.

Method Sample #	I	II	III	IV
101	0.52	0.19	0.45	0.40
102	0.36	0.16	0.34	0.31
103	0.38	0.16	0.38	0.34
104	0.34	0.15	0.37	0.33
105	0.39	0.17	0.37	0.34
106	0.38	0.16	0.40	0.37
110	0.48	0.21	0.40	0.36
111	0.25	0.15	0.31	0.28
112	0.38	0.18	0.36	0.33
113	0.37	0.16	0.35	0.32
114	0.38	0.15	0.32	0.29
115	0.41	0.17	0.36	0.32
119	0.30	0.17	0.38	0.35
120	0.37	0.16	0.37	0.33
121	0.34	0.17	0.42	0.38
122	0.29	0.18	0.42	0.39
123	0.28	0.17	0.41	0.37
124	0.32	0.19	0.44	0.40
128	0.22	0.13	0.35	0.32
129	0.33	0.15	0.36	0.32
130	0.36	0.17	0.38	0.34
131	0.25	0.16	0.38	0.35
132	0.34	0.16	0.40	0.36
133	0.19	0.13	0.33	0.31
201	0.31	0.26	0.44	0.39
202	0.36	0.29	0.43	0.39
203	0.43	0.24	0.37	0.33
204	0.25	0.26	0.40	0.36
205	0.31	0.24	0.39	0.37
206	0.38	0.22	0.40	0.37
207	0.32	0.13	0.29	0.27
208	0.30	0.14	0.26	0.24
209	0.31	0.13	0.28	0.25
210	0.22	0.12	0.24	0.21
211	0.23	0.11	0.25	0.22

Method Sample #	I	II	III	IV
212	0.16	0.14	0.28	0.25
213	0.31	0.16	0.40	0.37
214	0.37	0.20	0.41	0.38
215	0.34	0.19	0.40	0.36
216	0.42	0.21	0.42	0.38
217	0.33	0.20	0.42	0.38
218	0.35	0.21	0.42	0.37
219	0.24	0.14	0.34	0.30
220	0.33	0.13	0.33	0.29
221	0.38	0.16	0.36	0.32
222	0.28	0.13	0.28	0.25
223	0.26	0.13	0.32	0.29
224	0.27	0.15	0.35	0.31
301	0.50	0.20	0.32	0.30
302	0.46	0.24	0.37	0.34
303	0.36	0.19	0.33	0.31
304	0.52	0.21	0.34	0.32
305	0.46	0.21	0.35	0.34
306	0.16	0.09	0.22	0.21
307	0.27	0.11	0.24	0.23
308	0.20	0.11	0.24	0.23
309	0.20	0.12	0.27	0.25
310	0.18	0.10	0.23	0.21
311	0.23	0.11	0.24	0.21
312	0.23	0.15	0.32	0.29
314	0.33	0.16	0.34	0.32
315	0.24	0.13	0.33	0.29
317	0.38	0.17	0.34	0.29
318	0.34	0.17	0.34	0.27
319	0.19	0.10	0.25	0.22
320	0.20	0.10	0.29	0.23
321	0.26	0.13	0.30	0.27
322	0.22	0.12	0.25	0.22
323	0.26	0.14	0.27	0.25
324	0.31	0.15	0.37	0.34
401	0.36	0.18	0.26	0.24
402	0.38	0.20	0.26	0.24
403	0.28	0.16	0.24	0.22
404	0.31	0.16	0.24	0.22
405	0.29	0.15	0.26	0.22
406	0.27	0.16	0.22	0.20

Method Sample #	I	II	III	IV
407	0.20	0.10	0.19	0.16
408	0.17	0.09	0.18	0.16
409	0.24	0.11	0.18	0.17
410	0.16	0.09	0.17	0.15
411	0.18	0.10	0.16	0.15
412	0.19	0.10	0.19	0.17
413	0.22	0.11	0.26	0.23
414	0.28	0.15	0.26	0.24
415	0.29	0.12	0.26	0.23
416	0.31	0.15	0.26	0.23
417	0.19	0.12	0.24	0.22
418	0.29	0.15	0.24	0.22
419	0.17	0.10	0.18	0.17
420	0.23	0.11	0.19	0.17
421	0.24	0.12	0.20	0.18
422	0.22	0.11	0.18	0.16
423	0.24	0.13	0.20	0.18
424	0.22	0.11	0.18	0.16
501	0.24	0.13	0.20	0.19
502	0.23	0.12	0.18	0.17
503	0.27	0.12	0.19	0.17
504	0.20	0.10	0.16	0.15
505	0.26	0.13	0.19	0.18
506	0.23	0.12	0.19	0.18
507	0.22	0.10	0.18	0.16
508	0.22	0.10	0.17	0.16
509	0.16	0.09	0.15	0.15
510	0.20	0.09	0.16	0.15
511	0.15	0.08	0.14	0.13
512	0.21	0.11	0.18	0.18
513	0.24	0.10	0.20	0.19
514	0.25	0.12	0.22	0.20
515	0.19	0.09	0.17	0.16
516	0.22	0.11	0.20	0.19
517	0.32	0.14	0.22	0.20
518	0.21	0.10	0.18	0.17
519	0.19	0.09	0.17	0.15
520	0.16	0.08	0.16	0.14
521	0.23	0.13	0.21	0.19
522	0.23	0.13	0.20	0.19
523	0.18	0.10	0.17	0.15

Method Sample #	I	II	III	IV
524	0.20	0.11	0.18	0.16
601	0.21	0.11	0.18	0.16
602	0.23	0.11	0.20	0.17
603	0.24	0.13	0.20	0.18
604	0.25	0.13	0.17	0.15
605	0.24	0.13	0.20	0.18
606	0.17	0.09	0.15	0.13
610	0.19	0.12	0.18	0.17
611	0.21	0.13	0.19	0.17
612	0.21	0.11	0.15	0.14
613	0.23	0.12	0.17	0.16
614	0.17	0.10	0.15	0.13
615	0.18	0.12	0.18	0.17
616	0.23	0.11	0.17	0.15
617	0.18	0.10	0.15	0.14
618	0.24	0.09	0.16	0.14
619	0.37	0.14	0.21	0.19
620	0.29	0.13	0.18	0.16
621	0.34	0.14	0.18	0.17
625	0.28	0.10	0.16	0.15
626	0.25	0.15	0.20	0.19
627	0.19	0.11	0.18	0.16
628	0.35	0.15	0.20	0.18
629	0.33	0.15	0.19	0.17
630	0.27	0.12	0.16	0.15

Appendix. 11. Calcium concentration (%) of Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard; V = Perchloric acid digest sample/Chemical standard.

Method Sample #	I	II	III	IV	V
101	0.48	0.49	0.59	0.57	0.48
102	0.37	0.45	0.48	0.48	0.43
103	0.36	0.42	0.48	0.46	0.48
104	0.39	0.46	0.51	0.50	0.54
105	0.42	0.49	0.52	0.50	0.54
106	0.38	0.46	0.51	0.50	0.47
110	0.38	0.44	0.46	0.46	0.47
111	0.25	0.42	0.41	0.42	0.44
112	0.34	0.46	0.45	0.46	0.48
113	0.33	0.39	0.39	0.41	0.41
114	0.32	0.36	0.34	0.35	0.37
115	0.34	0.39	0.39	0.39	0.42
119	0.27	0.43	0.42	0.43	0.54
120	0.38	0.44	0.46	0.46	0.48
121	0.35	0.47	0.51	0.50	0.55
122	0.21	0.37	0.39	0.39	0.42
123	0.22	0.37	0.40	0.40	0.42
124	0.21	0.35	0.38	0.37	0.38
128	0.20	0.34	0.36	0.36	0.41
129	0.29	0.36	0.36	0.36	0.41
130	0.24	0.31	0.33	0.33	0.37
131	0.17	0.31	0.36	0.31	0.37
132	0.25	0.35	0.39	0.35	0.39
133	0.18	0.34	0.39	0.36	0.40
201	0.15	0.40	0.48	0.44	0.50
202	0.19	0.45	0.53	0.50	0.55
203	0.30	0.47	0.52	0.48	0.53
204	0.12	0.39	0.43	0.40	0.47
205	0.17	0.41	0.51	0.48	0.50
206	0.24	0.40	0.45	0.44	0.49
207	0.42	0.47	0.52	0.50	0.56
208	0.31	0.40	0.42	0.40	0.43
209	0.36	0.41	0.47	0.46	0.47
210	0.28	0.42	0.44	0.43	0.45
211	0.27	0.37	0.41	0.40	0.44

Method Sample #	I	II	III	IV	V
212	0.17	0.44	0.46	0.45	0.47
213	0.30	0.44	0.53	0.51	0.52
214	0.32	0.49	0.53	0.52	0.56
215	0.27	0.44	0.50	0.50	0.49
216	0.27	0.41	0.41	0.43	0.65
217	0.21	0.39	0.42	0.43	0.42
218	0.22	0.40	0.41	0.42	0.41
219	0.20	0.33	0.33	0.33	0.35
220	0.28	0.28	0.30	0.32	0.34
221	0.23	0.25	0.28	0.28	0.30
222	0.22	0.28	0.29	0.28	0.31
223	0.21	0.28	0.32	0.32	0.33
224	0.16	0.24	0.27	0.27	0.28
301	0.39	0.43	0.46	0.46	0.28
302	0.24	0.37	0.43	0.44	0.48
303	0.26	0.40	0.45	0.48	0.43
304	0.36	0.37	0.39	0.40	0.46
305	0.34	0.41	0.45	0.47	0.40
306	0.26	0.40	0.43	0.47	0.39
307	0.34	0.39	0.41	0.45	0.46
308	0.24	0.41	0.42	0.46	0.47
309	0.21	0.40	0.42	0.42	0.43
310	0.21	0.39	0.41	0.40	0.42
311	0.31	0.39	0.45	0.43	0.41
312	0.22	0.49	0.45	0.45	0.45
314	0.32	0.46	0.51	0.52	0.50
315	0.23	0.40	0.44	0.43	0.45
317	0.28	0.36	0.39	0.32	0.42
318	0.27	0.40	0.43	0.32	0.46
319	0.21	0.33	0.34	0.31	0.38
320	0.23	0.33	0.38	0.26	0.37
321	0.19	0.27	0.27	0.29	0.29
322	0.19	0.30	0.29	0.30	0.35
323	0.16	0.26	0.24	0.26	0.29
324	0.23	0.30	0.38	0.40	0.34
401	0.26	0.37	0.35	0.38	0.41
402	0.27	0.40	0.36	0.38	0.43
403	0.23	0.36	0.35	0.36	0.39
404	0.21	0.30	0.29	0.31	0.33
405	0.22	0.33	0.34	0.35	0.38
406	0.19	0.34	0.31	0.32	0.37

Method Sample #	I	II	III	IV	V
407	0.24	0.34	0.32	0.31	0.41
408	0.27	0.41	0.40	0.40	0.41
409	0.32	0.38	0.35	0.35	0.43
410	0.27	0.40	0.37	0.37	0.41
411	0.22	0.33	0.30	0.30	0.34
412	0.25	0.36	0.36	0.37	0.36
413	0.28	0.42	0.42	0.43	0.45
414	0.28	0.43	0.43	0.43	0.45
415	0.30	0.36	0.38	0.38	0.40
416	0.24	0.36	0.33	0.34	0.37
417	0.17	0.35	0.36	0.36	0.36
418	0.22	0.34	0.34	0.33	0.37
419	0.19	0.31	0.29	0.28	0.32
420	0.22	0.30	0.28	0.27	0.30
421	0.19	0.28	0.26	0.25	0.29
422	0.21	0.30	0.27	0.26	0.30
423	0.20	0.30	0.29	0.28	0.30
424	0.18	0.25	0.22	0.21	0.26
501	0.20	0.31	0.34	0.33	0.34
502	0.20	0.29	0.30	0.30	0.33
503	0.24	0.31	0.33	0.35	0.34
504	0.17	0.25	0.25	0.26	0.29
505	0.19	0.27	0.28	0.29	0.37
506	0.18	0.28	0.30	0.31	0.32
507	0.23	0.29	0.32	0.33	0.36
508	0.27	0.34	0.38	0.39	0.40
509	0.15	0.25	0.22	0.24	0.29
510	0.26	0.34	0.35	0.37	0.40
511	0.16	0.23	0.22	0.23	0.28
512	0.24	0.33	0.35	0.37	0.43
513	0.24	0.32	0.37	0.38	0.36
514	0.22	0.32	0.36	0.38	0.40
515	0.23	0.34	0.35	0.35	0.42
516	0.20	0.29	0.29	0.31	0.37
517	0.25	0.33	0.33	0.34	0.40
518	0.17	0.27	0.29	0.30	0.34
519	0.14	0.20	0.18	0.19	0.26
520	0.15	0.23	0.25	0.26	0.31
521	0.14	0.24	0.22	0.23	0.28
522	0.16	0.26	0.24	0.26	0.30
523	0.14	0.24	0.23	0.24	0.29

Method Sample #	I	II	III	IV	V
524	0.14	0.25	0.30	0.25	0.32
601	0.23	0.35	0.31	0.32	0.40
602	0.23	0.32	0.32	0.32	0.40
603	0.23	0.34	0.34	0.35	0.40
604	0.18	0.27	0.22	0.24	0.41
605	0.20	0.33	0.31	0.33	0.39
606	0.19	0.30	0.28	0.29	0.36
610	0.20	0.39	0.41	0.40	0.48
611	0.24	0.43	0.44	0.43	0.49
612	0.20	0.31	0.28	0.28	0.34
613	0.19	0.28	0.27	0.27	0.33
614	0.15	0.26	0.25	0.25	0.33
615	0.16	0.31	0.32	0.32	0.37
616	0.27	0.37	0.36	0.34	0.40
617	0.18	0.32	0.31	0.30	0.36
618	0.33	0.35	0.40	0.39	0.44
619	0.36	0.39	0.41	0.39	0.42
620	0.26	0.32	0.33	0.32	0.35
621	0.29	0.34	0.32	0.31	0.38
625	0.24	0.25	0.26	0.24	0.31
626	0.16	0.26	0.27	0.26	0.32
627	0.19	0.31	0.31	0.30	0.34
628	0.24	0.27	0.27	0.26	0.30
629	0.25	0.32	0.31	0.30	0.34
630	0.21	0.28	0.26	0.25	0.30

Appendix. 12. Magnesium concentration (%) of Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard; V = Perchloric acid digest sample/Chemical standard.

Method Sample #	I	II	III	IV	V
101	0.22	0.24	0.30	0.29	0.23
102	0.18	0.22	0.26	0.25	0.24
103	0.17	0.21	0.27	0.26	0.24
104	0.16	0.19	0.22	0.22	0.20
105	0.18	0.21	0.24	0.23	0.23
106	0.16	0.20	0.24	0.23	0.20
110	0.13	0.15	0.17	0.16	0.15
111	0.09	0.15	0.16	0.16	0.16
112	0.11	0.15	0.16	0.16	0.16
113	0.12	0.14	0.16	0.16	0.15
114	0.12	0.14	0.14	0.14	0.13
115	0.13	0.15	0.16	0.16	0.15
119	0.15	0.25	0.28	0.29	0.32
120	0.20	0.26	0.31	0.31	0.29
121	0.18	0.26	0.31	0.31	0.31
122	0.12	0.22	0.25	0.25	0.25
123	0.12	0.20	0.24	0.24	0.23
124	0.13	0.22	0.26	0.26	0.23
128	0.11	0.18	0.21	0.21	0.21
129	0.15	0.18	0.21	0.20	0.21
130	0.15	0.19	0.22	0.22	0.22
131	0.11	0.19	0.22	0.21	0.21
132	0.15	0.19	0.23	0.22	0.21
133	0.09	0.17	0.20	0.20	0.18
201	0.09	0.21	0.26	0.25	0.26
202	0.09	0.21	0.25	0.24	0.25
203	0.13	0.21	0.25	0.24	0.24
204	0.07	0.20	0.24	0.23	0.25
205	0.08	0.19	0.24	0.24	0.23
206	0.13	0.19	0.24	0.24	0.24
207	0.12	0.13	0.15	0.15	0.15
208	0.11	0.14	0.15	0.15	0.14
209	0.11	0.13	0.16	0.16	0.15
210	0.08	0.12	0.13	0.13	0.13
211	0.08	0.10	0.13	0.13	0.12

Method / Sample #	I	II	III	IV	V
212	0.06	0.14	0.15	0.15	0.14
213	0.16	0.24	0.31	0.31	0.29
214	0.16	0.25	0.30	0.30	0.29
215	0.16	0.25	0.30	0.30	0.28
216	0.17	0.24	0.26	0.26	0.39
217	0.13	0.23	0.27	0.27	0.25
218	0.13	0.24	0.27	0.26	0.24
219	0.10	0.16	0.18	0.18	0.17
220	0.17	0.17	0.20	0.19	0.18
221	0.14	0.16	0.20	0.19	0.18
222	0.12	0.16	0.17	0.17	0.17
223	0.12	0.17	0.19	0.19	0.18
224	0.12	0.18	0.21	0.21	0.19
301	0.18	0.21	0.24	0.24	0.19
302	0.12	0.19	0.23	0.24	0.23
303	0.12	0.19	0.23	0.24	0.22
304	0.18	0.19	0.20	0.21	0.22
305	0.15	0.19	0.22	0.23	0.20
306	0.07	0.11	0.12	0.13	0.20
307	0.12	0.14	0.16	0.16	0.13
308	0.08	0.13	0.15	0.15	0.16
309	0.08	0.16	0.17	0.18	0.14
310	0.08	0.14	0.16	0.15	0.16
311	0.10	0.14	0.15	0.15	0.15
312	0.12	0.28	0.27	0.27	0.15
314	0.17	0.24	0.29	0.29	0.26
315	0.13	0.23	0.27	0.27	0.25
317	0.18	0.23	0.26	0.25	0.26
318	0.15	0.23	0.27	0.24	0.26
319	0.12	0.18	0.20	0.19	0.21
320	0.13	0.18	0.22	0.20	0.20
321	0.12	0.17	0.18	0.19	0.18
322	0.10	0.16	0.17	0.18	0.18
323	0.12	0.18	0.18	0.19	0.20
324	0.14	0.19	0.26	0.26	0.21
401	0.14	0.20	0.20	0.21	0.21
402	0.15	0.21	0.21	0.21	0.23
403	0.12	0.19	0.19	0.20	0.20
404	0.12	0.17	0.17	0.17	0.18
405	0.12	0.18	0.20	0.20	0.20
406	0.10	0.18	0.18	0.18	0.19

Method Sample #	I	II	III	IV	V
407	0.10	0.15	0.15	0.15	0.16
408	0.09	0.14	0.14	0.14	0.15
409	0.12	0.15	0.15	0.16	0.16
410	0.08	0.12	0.12	0.12	0.12
411	0.09	0.13	0.13	0.13	0.16
412	0.09	0.13	0.14	0.14	0.14
413	0.15	0.23	0.25	0.25	0.24
414	0.16	0.25	0.26	0.26	0.25
415	0.18	0.23	0.26	0.25	0.25
416	0.16	0.23	0.23	0.23	0.23
417	0.11	0.22	0.24	0.24	0.23
418	0.14	0.22	0.23	0.23	0.22
419	0.10	0.16	0.17	0.17	0.17
420	0.13	0.18	0.18	0.18	0.17
421	0.12	0.17	0.17	0.17	0.17
422	0.13	0.17	0.17	0.17	0.18
423	0.12	0.17	0.18	0.18	0.17
424	0.12	0.17	0.17	0.16	0.17
501	0.10	0.16	0.18	0.18	0.17
502	0.11	0.17	0.19	0.19	0.11
503	0.13	0.16	0.18	0.19	0.18
504	0.09	0.13	0.14	0.15	0.15
505	0.10	0.15	0.17	0.17	0.19
506	0.09	0.15	0.17	0.17	0.17
507	0.09	0.12	0.15	0.15	0.15
508	0.09	0.12	0.15	0.15	0.14
509	0.07	0.13	0.13	0.14	0.15
510	0.08	0.11	0.12	0.13	0.13
511	0.06	0.09	0.10	0.11	0.12
512	0.09	0.14	0.16	0.17	0.18
513	0.16	0.21	0.26	0.27	0.17
514	0.14	0.21	0.25	0.26	0.25
515	0.12	0.18	0.20	0.20	0.21
516	0.13	0.19	0.20	0.21	0.21
517	0.14	0.18	0.20	0.20	0.20
518	0.11	0.17	0.21	0.21	0.21
519	0.08	0.12	0.13	0.13	0.13
520	0.10	0.15	0.18	0.18	0.19
521	0.09	0.15	0.16	0.16	0.16
522	0.10	0.15	0.16	0.17	0.17
523	0.09	0.16	0.17	0.17	0.17

Method Sample #	I	II	III	IV	V
524	0.08	0.14	0.18	0.16	0.17
601	0.12	0.18	0.19	0.19	0.20
602	0.10	0.14	0.16	0.17	0.18
603	0.10	0.15	0.17	0.17	0.17
604	0.09	0.13	0.12	0.12	0.18
605	0.10	0.17	0.18	0.19	0.20
606	0.08	0.13	0.14	0.14	0.16
610	0.07	0.13	0.14	0.15	0.15
611	0.08	0.14	0.15	0.15	0.16
612	0.09	0.14	0.14	0.14	0.14
613	0.08	0.11	0.12	0.12	0.12
614	0.06	0.12	0.12	0.13	0.14
615	0.07	0.14	0.16	0.16	0.17
616	0.15	0.23	0.24	0.23	0.23
617	0.09	0.17	0.18	0.18	0.18
618	0.19	0.21	0.26	0.26	0.26
619	0.22	0.24	0.26	0.26	0.26
620	0.15	0.20	0.21	0.20	0.21
621	0.17	0.21	0.21	0.21	0.23
625	0.14	0.15	0.16	0.16	0.17
626	0.08	0.14	0.16	0.16	0.17
627	0.10	0.16	0.18	0.17	0.17
628	0.14	0.17	0.17	0.17	0.17
629	0.14	0.18	0.19	0.18	0.18
630	0.12	0.15	0.16	0.15	0.16

Appendix. 13. Manganese concentration (ppm) of Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard.

Method Sample #	I	II	III	IV
101	143.07	164.20	211.60	169.41
102	99.84	141.20	174.80	139.27
103	119.68	150.80	217.60	173.08
104	82.73	105.20	131.20	105.11
105	97.37	127.60	150.00	118.97
106	108.96	141.80	179.20	146.11
110	107.96	140.00	221.20	176.73
111	121.70	224.20	372.80	307.00
112	118.77	200.00	301.20	248.15
113	121.17	151.00	244.80	203.19
114	140.02	161.20	268.00	222.00
115	163.04	239.40	331.20	267.53
119	92.64	219.40	297.20	242.73
120	178.50	252.00	357.60	288.14
121	108.79	189.60	266.40	220.05
122	97.69	187.80	236.00	197.56
123	95.76	178.60	254.40	205.62
124	81.82	167.80	233.60	189.89
128	151.34	235.60	408.00	332.67
129	177.22	245.00	389.20	314.76
130	148.62	218.40	326.40	262.25
131	119.90	207.40	282.00	234.68
132	211.10	268.00	394.40	320.22
133	114.62	229.20	362.00	305.29
201	47.00	120.80	158.80	128.15
202	56.86	133.20	175.20	144.91
203	87.08	135.60	162.80	130.39
204	34.78	93.80	110.40	89.61
205	49.90	118.00	156.40	134.06
206	64.50	105.80	133.60	112.92
207	138.44	162.20	232.40	196.89
208	137.59	187.00	301.60	252.26
209	145.06	163.60	272.80	228.98
210	90.85	126.80	185.20	153.78
211	95.99	124.40	204.40	173.13

Method Sample #	I	II	III	IV
212	58.62	154.80	220.40	182.64
213	89.86	160.40	225.20	186.74
214	95.94	176.40	228.80	191.22
215	100.05	178.20	234.00	192.40
216	110.20	174.60	190.00	159.53
217	80.39	153.20	182.40	153.44
218	82.19	167.80	189.60	156.05
219	105.15	147.40	272.80	225.37
220	230.27	187.40	357.60	297.92
221	127.88	148.20	228.40	185.88
222	125.61	167.60	258.00	210.81
223	123.12	164.00	284.80	240.05
224	103.61	130.80	244.80	204.45
301	106.68	126.20	141.60	118.18
302	71.82	113.60	148.00	125.79
303	61.98	97.60	129.60	114.03
304	106.70	107.40	112.80	97.29
305	94.21	112.80	132.40	113.52
306	56.40	106.40	0.00	112.68
307	100.28	146.60	297.20	261.01
308	151.88	175.60	287.60	252.73
309	85.21	146.00	240.40	211.46
310	81.08	135.20	291.60	245.88
311	76.61	143.00	209.60	174.09
312	108.46	141.00	215.60	176.68
314	95.08	136.40	174.00	147.32
315	79.52	135.60	169.20	138.59
317	106.89	138.20	155.60	118.50
318	99.11	163.80	192.80	136.31
319	134.72	175.00	412.80	332.95
320	127.48	146.20	415.20	291.62
321	121.83	120.80	340.80	282.22
322	100.30	115.00	283.20	234.35
323	108.30	112.80	246.00	212.99
324	145.24	163.00	450.00	379.57
401	83.57	112.40	118.80	99.87
402	82.93	116.40	118.00	99.25
403	60.13	95.20	105.20	87.01
404	61.79	84.60	88.00	74.23
405	58.84	88.00	104.00	84.40
406	51.63	93.20	97.60	79.62

Method Sample #	I	II	III	IV
407	87.06	100.20	216.80	176.67
408	83.62	131.80	212.00	173.36
409	108.16	117.20	238.80	196.49
410	103.29	156.60	250.00	206.30
411	101.44	144.60	204.80	170.41
412	92.34	142.60	214.40	178.89
413	78.96	113.00	154.80	127.15
414	78.19	136.80	153.60	128.65
415	90.79	119.40	147.60	120.12
416	85.91	128.80	141.20	116.06
417	63.19	117.80	141.60	117.55
418	76.42	124.40	140.00	115.73
419	104.05	157.00	466.40	386.43
420	116.33	169.00	367.60	300.86
421	106.72	147.40	280.40	233.75
422	117.73	187.40	308.00	256.04
423	99.84	138.80	270.80	223.29
424	106.32	110.60	231.60	190.97
501	58.91	95.60	120.80	100.93
502	52.24	79.20	91.20	76.02
503	67.73	90.40	100.00	81.98
504	47.69	70.20	72.80	61.26
505	50.55	74.80	84.80	70.93
506	46.25	84.00	98.40	81.08
507	68.86	98.00	180.80	150.06
508	79.20	121.20	175.20	144.53
509	53.09	87.80	158.80	138.61
510	93.96	151.40	287.60	246.62
511	58.68	88.00	137.60	117.96
512	83.53	146.20	206.40	177.14
513	72.28	101.80	131.20	111.45
514	69.66	118.60	150.80	128.01
515	48.75	79.00	99.20	83.45
516	60.11	93.00	128.00	111.32
517	77.44	95.00	124.40	106.01
518	61.28	108.20	137.60	116.31
519	79.99	128.00	262.80	218.31
520	82.85	142.20	437.60	365.82
521	76.86	148.60	269.20	230.99
522	73.66	121.00	247.20	212.81
523	82.77	160.60	324.40	266.18

Method Sample #	I	II	III	IV
524	65.75	101.00	146.00	200.94
601	65.71	125.20	130.40	107.68
602	46.44	77.00	100.40	83.68
603	52.45	94.80	121.60	101.03
604	50.21	75.20	77.20	62.91
605	45.01	86.20	102.80	85.70
606	50.64	97.40	122.40	100.52
610	56.89	130.80	211.60	175.39
611	69.59	146.40	222.40	181.69
612	63.97	131.00	192.40	156.69
613	77.61	154.80	261.60	213.60
614	42.60	84.80	146.80	122.55
615	54.38	137.00	218.80	181.08
616	62.62	117.60	124.80	101.21
617	45.54	98.00	106.80	87.90
618	77.25	99.00	130.40	108.53
619	83.53	107.20	137.20	113.42
620	74.58	111.80	141.60	116.04
621	98.21	133.00	141.20	116.78
625	108.12	169.40	302.00	247.22
626	97.05	195.60	619.20	516.94
627	66.73	116.00	238.00	193.30
628	94.33	112.20	217.60	178.27
629	117.81	218.80	399.60	334.77
630	94.17	166.80	266.00	223.08

Appendix. 14. Copper concentration (%) in Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard; V = Perchloric acid digest sample/Chemical standard.

Method Sample #	I	II	II	IV	V
101	5.58	7.20	10.80	10.05	4.00
102	2.81	5.20	8.40	7.47	4.00
103	2.47	4.60	10.00	9.19	4.00
104	3.83	6.00	8.40	7.98	2.00
105	2.86	5.40	8.40	7.77	2.00
106	2.36	5.00	10.00	9.44	1.00
110	1.99	3.40	6.40	6.12	5.00
111	1.69	3.60	6.00	5.62	6.00
112	1.68	3.40	6.80	6.48	6.00
113	2.49	4.20	5.60	5.49	6.00
114	2.14	3.60	4.80	4.81	5.00
115	1.70	2.80	6.80	6.45	5.00
119	1.59	4.00	10.00	9.62	11.00
120	2.47	4.00	14.40	13.64	10.00
121	2.63	4.80	11.20	10.38	13.00
122	1.65	5.00	8.00	7.61	8.00
123	1.51	4.20	11.20	10.44	8.00
124	2.05	5.60	8.80	8.47	7.00
128	1.37	3.00	6.00	5.70	4.00
129	2.05	3.80	6.40	6.13	4.00
130	2.33	4.40	6.40	6.06	4.00
131	1.96	4.80	7.20	5.94	3.00
132	1.66	3.80	6.40	5.56	2.00
133	0.73	3.20	5.60	4.98	1.00
201	1.26	5.80	11.20	9.83	6.00
202	1.62	6.40	8.80	7.89	4.00
203	2.05	5.60	8.80	7.44	10.00
204	1.69	6.60	8.40	7.41	9.00
205	1.09	5.20	8.40	7.89	9.00
206	1.69	5.20	7.20	6.91	8.00
207	1.63	3.60	4.80	4.47	5.00
208	1.37	3.40	4.40	4.14	5.00
209	0.77	2.20	4.40	4.13	4.00
210	1.39	3.60	4.40	4.03	4.00
211	1.41	3.20	4.00	3.51	4.00

Method Sample #	I	II	II	IV	V
212	0.38	3.40	4.00	3.78	3.00
213	0.93	3.20	9.60	9.26	10.00
214	1.63	5.20	9.60	9.18	10.00
215	1.08	4.40	9.60	9.13	9.00
216	3.67	7.00	8.00	7.99	13.00
217	2.07	6.40	8.80	8.50	8.00
218	2.31	6.80	8.00	7.76	8.00
219	1.30	3.60	4.00	3.92	6.00
220	3.31	3.00	4.40	4.32	3.00
221	2.94	3.40	4.40	4.06	7.00
222	2.20	3.20	4.40	3.63	5.00
223	1.73	3.00	4.00	4.11	5.00
224	2.07	3.40	4.40	4.42	6.00
301	3.04	4.80	6.80	6.23	7.00
302	2.32	4.80	6.80	6.84	6.00
303	1.73	3.80	6.40	6.89	7.00
304	4.49	5.20	6.40	5.98	6.00
305	3.42	4.40	6.00	6.02	5.00
306	1.24	2.20	3.20	3.69	3.00
307	1.17	2.20	4.00	4.37	3.00
308	0.56	2.20	4.00	4.44	2.00
309	0.76	2.80	6.00	5.63	3.00
310	0.58	2.60	4.80	4.53	5.00
311	0.95	2.60	7.20	6.58	4.00
312	1.64	9.00	9.60	9.58	9.00
314	1.74	5.60	9.60	9.73	8.00
315	2.01	6.00	9.60	9.09	9.00
317	4.41	6.40	9.60	7.48	13.00
318	2.98	6.20	8.80	5.76	11.00
319	0.71	2.00	3.20	2.68	5.00
320	1.23	2.60	4.80	1.73	5.00
321	1.46	2.40	3.60	3.46	4.00
322	1.38	2.80	4.00	3.83	4.00
323	1.32	2.80	3.60	3.70	4.00
324	1.43	2.60	4.80	4.62	4.00
401	2.47	3.80	4.80	5.11	6.00
402	2.14	4.00	5.20	4.92	7.00
403	1.58	3.60	4.80	4.61	4.00
404	1.96	3.60	4.40	4.39	4.00
405	1.66	3.60	4.40	4.01	4.00
406	1.20	4.00	4.40	4.05	3.00

Method Sample #	I	II	II	IV	V
407	1.12	2.00	4.40	3.79	2.00
408	0.81	2.20	3.60	3.60	2.00
409	1.73	2.40	4.40	4.34	1.00
410	0.56	1.80	3.60	3.27	6.00
411	0.95	2.20	4.80	4.43	6.00
412	1.22	2.40	2.40	2.23	10.00
413	3.06	6.00	8.40	7.83	10.00
414	2.35	6.80	8.80	8.36	8.00
415	2.17	5.60	8.00	7.77	8.00
416	3.64	7.00	9.20	8.63	9.00
417	2.49	6.20	10.80	10.11	8.00
418	2.92	6.20	7.60	7.08	8.00
419	0.62	1.80	2.40	2.27	3.00
420	1.41	3.00	4.40	4.13	3.00
421	1.42	3.00	3.20	3.04	4.00
422	1.21	2.80	2.80	2.40	3.00
423	1.56	3.40	3.20	3.07	3.00
424	1.76	3.20	3.20	2.94	3.00
501	1.43	2.80	4.00	3.74	3.00
502	1.42	2.80	4.40	4.20	3.00
503	1.36	2.60	6.40	5.85	3.00
504	1.55	2.80	6.00	5.89	2.00
505	1.57	3.00	6.40	5.89	3.00
506	0.71	2.40	8.00	7.45	2.00
507	1.15	2.20	5.20	5.37	2.00
508	0.85	1.80	6.00	5.82	1.00
509	0.94	2.20	2.80	2.58	7.00
510	0.58	1.40	2.40	2.25	4.00
511	0.97	1.80	2.40	2.35	1.20
512	0.85	2.00	3.60	3.75	4.00
513	1.56	4.00	11.20	10.62	7.00
514	1.40	4.40	8.80	8.57	7.00
515	1.63	4.00	20.00	19.10	8.00
516	2.71	5.20	8.80	8.60	10.00
517	2.85	4.80	14.00	13.58	6.00
518	1.00	3.40	10.00	9.82	6.00
519	0.62	2.00	8.40	7.82	4.00
520	0.51	1.80	10.80	10.43	5.00
521	0.85	2.80	13.60	13.26	4.00
522	1.05	2.60	6.40	6.38	4.00
523	0.87	2.60	2.40	2.25	3.00

Method Sample #	I	II	II	IV	V
524	0.96	2.60	2.40	3.12	4.00
601	0.89	2.60	2.40	2.44	5.00
602	0.69	2.40	2.80	3.00	4.00
603	0.84	2.40	2.80	3.12	4.00
604	1.55	3.00	4.40	4.56	4.00
605	0.83	2.80	2.80	2.94	3.00
606	0.57	2.20	2.80	2.88	3.00
610	0.46	1.80	3.20	2.92	3.00
611	0.60	2.20	3.60	3.24	4.00
612	1.03	2.40	4.80	4.54	2.00
613	0.50	1.40	4.00	3.42	2.00
614	0.64	2.20	4.00	3.80	2.00
615	0.45	2.00	2.80	2.80	4.00
616	1.46	3.80	5.60	4.98	7.00
617	0.72	3.00	4.00	3.61	5.00
618	1.24	2.40	4.00	3.80	5.00
619	3.78	5.00	6.40	6.00	7.00
620	2.42	3.80	12.40	11.55	6.00
621	2.28	4.00	4.80	4.19	13.00
625	1.10	2.20	9.60	8.77	4.00
626	0.31	1.40	4.80	4.65	5.00
627	1.04	2.60	5.20	4.84	5.00
628	1.77	2.80	3.60	3.03	4.00
629	1.08	2.40	2.80	2.32	4.00
630	1.29	2.80	2.80	2.39	4.00

Appendix. 15. Boron concentration (%) in Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard.

Method Sample #	I	II	III	IV
101	4.32	4.80	7.60	11.29
102	3.67	4.60	5.60	9.43
103	4.01	5.00	6.40	10.14
104	3.43	4.20	4.80	9.31
105	4.09	4.60	5.60	10.01
106	3.54	4.80	6.00	10.20
110	2.28	2.40	3.20	7.79
111	1.60	2.80	2.80	2.97
112	2.31	2.80	3.20	3.45
113	1.87	3.00	3.20	3.42
114	1.89	2.40	2.80	3.00
115	2.34	3.20	3.20	3.43
119	1.91	4.20	4.80	4.28
120	4.48	5.20	7.60	6.80
121	2.31	4.20	5.60	4.74
122	2.37	4.80	5.20	4.75
123	2.09	4.20	4.80	3.81
124	1.68	3.60	4.00	3.82
128	2.58	4.40	5.60	4.72
129	3.60	4.80	5.60	4.67
130	3.19	4.60	5.20	4.71
131	2.42	4.60	6.40	4.65
132	3.65	5.20	6.80	4.75
133	2.06	4.40	5.60	3.84
201	1.35	4.40	6.00	4.69
202	1.61	4.20	5.20	3.94
203	2.28	4.20	5.20	3.47
204	0.94	4.20	4.80	3.43
205	1.40	4.00	5.20	4.08
206	1.84	4.00	4.40	3.61
207	1.59	2.40	2.40	1.82
208	1.85	3.00	2.40	1.37
209	1.84	2.60	2.40	1.79
210	1.17	2.20	2.00	1.78

Method Sample #	I	II	III	IV
211	1.13	2.40	2.40	1.34
212	0.70	2.80	2.40	1.78
213	2.09	3.60	4.40	3.58
214	2.30	3.80	4.00	3.06
215	1.85	3.60	4.40	4.01
216	2.59	3.60	3.60	4.07
217	1.88	3.20	3.20	4.01
218	1.85	3.80	4.40	4.01
219	2.32	4.00	4.40	3.55
220	3.34	3.60	4.00	4.02
221	2.80	3.40	4.00	3.56
222	2.91	3.80	4.00	8.27
223	0.96	3.80	4.00	4.00
224	0.95	3.60	4.00	4.00
301	3.38	4.00	4.40	3.60
302	0.94	3.60	4.40	4.10
303	0.94	3.60	4.40	4.09
304	3.28	3.60	3.20	3.61
305	0.95	4.00	4.40	3.63
306	0.94	2.00	-2.00	2.26
307	0.72	2.60	2.00	2.27
308	0.71	2.40	2.00	2.27
309	0.73	2.40	2.40	2.30
310	0.74	2.00	8.00	1.82
311	0.72	2.40	8.00	1.39
312	0.74	4.00	9.20	2.75
314	0.74	3.20	8.80	2.72
315	0.73	3.20	8.80	2.30
317	0.73	2.60	9.20	-4.53
318	0.74	2.80	8.80	-4.61
319	0.72	4.40	4.40	8.73
320	0.70	4.40	9.60	-4.52
321	0.73	4.00	4.40	7.89
322	0.70	4.20	3.60	7.47
323	0.74	3.80	3.60	7.33
324	0.73	4.20	5.60	9.38
401	0.73	4.40	4.80	8.94
402	0.70	4.60	4.40	8.28
403	0.73	4.80	4.40	8.35
404	0.70	4.00	4.00	7.73

Method Sample #	I	II	III	IV
405	0.71	4.60	4.80	8.60
406	0.73	4.20	3.60	7.44
407	0.73	3.80	3.60	2.57
408	0.71	3.40	3.20	1.74
409	0.72	3.80	3.60	2.61
410	0.72	3.20	2.80	2.17
411	0.72	3.20	2.80	2.13
412	0.71	3.60	3.20	2.68
413	0.74	4.80	4.00	3.90
414	0.74	5.20	4.80	3.94
415	0.73	4.60	4.00	3.49
416	0.71	5.20	4.00	3.90
417	0.73	4.60	6.00	4.53
418	0.73	4.80	4.80	3.57
419	0.74	6.40	7.20	4.73
420	4.56	6.40	5.60	4.35
421	0.73	5.80	6.00	4.20
422	4.54	6.40	5.60	4.49
423	0.72	6.00	6.00	4.15
424	0.72	5.80	5.20	3.99
501	0.95	3.60	5.60	4.28
502	0.94	3.60	4.00	2.96
503	0.93	3.60	5.20	3.95
504	0.95	3.20	4.40	3.42
505	0.92	0.20	5.20	3.89
506	0.95	0.20	4.00	3.45
507	0.93	0.20	3.60	2.65
508	0.94	0.20	2.80	1.73
509	0.91	0.20	2.40	1.72
510	0.91	0.20	3.20	2.13
511	0.94	0.20	2.80	1.74
512	0.93	0.20	5.60	4.70
513	0.96	0.20	4.40	3.45
514	0.98	0.20	4.00	3.05
515	0.98	0.20	3.60	2.54
516	0.96	0.20	4.00	3.01
517	0.97	0.20	4.00	3.00
518	0.97	0.20	4.00	3.47
519	0.97	0.20	4.00	2.57
520	0.98	0.20	3.60	2.96

Method Sample #	I	II	III	IV
521	0.97	0.20	3.60	3.40
522	0.95	0.20	4.40	3.43
523	0.96	0.20	3.60	9.19
524	0.97	0.20	4.80	8.75
601	3.90	5.20	4.80	10.06
602	3.90	5.00	4.80	10.06
603	0.97	4.60	4.80	10.06
604	3.57	5.40	4.40	9.63
605	0.95	4.80	4.80	10.06
606	0.97	4.40	3.60	9.19
610	0.97	0.20	4.00	7.13
611	0.94	0.20	4.00	6.75
612	0.96	0.20	2.80	6.37
613	0.98	0.20	2.40	6.37
614	0.95	0.20	2.80	7.13
615	0.95	3.40	4.40	7.50
616	0.97	3.20	3.20	6.75
617	0.96	3.40	3.20	7.13
618	0.93	3.00	4.00	7.50
619	3.83	3.80	4.40	3.50
620	0.95	3.80	4.00	3.06
621	3.31	3.80	4.00	3.06
625	6.43	6.40	8.40	7.44
626	4.32	6.80	8.40	6.56
627	4.37	6.80	8.00	6.13
628	4.36	5.20	6.00	4.81
629	5.08	6.20	8.40	7.52
630	4.51	5.60	6.40	5.31

Appendix. 16. Zinc concentration (%) in Tall fescue determined using different standardization and digestion methods.
Method: I = Dry ashed sample/Dry ashed standard; II = Dry ashed sample/Chemical standard; III = Microwave digest sample/Chemical standard; IV = Microwave digest sample/Microwave digest standard; V = Perchloric acid digest sample/Chemical standard.

Method Sample #	I	II	III	IV	V
101	33.63	34.00	43.60	37.74	35.00
102	22.98	29.00	60.80	52.67	30.10
103	21.45	27.80	36.40	31.21	32.60
104	22.78	27.40	32.00	27.84	26.70
105	24.34	30.00	33.60	29.17	27.70
106	19.15	26.20	32.00	28.30	25.60
110	18.51	22.20	25.20	21.72	20.10
111	12.90	22.20	33.20	29.23	21.90
112	15.95	22.60	25.60	22.78	23.40
113	18.26	21.60	24.80	22.72	21.40
114	16.96	19.20	30.40	27.28	17.10
115	15.73	18.80	23.20	20.29	18.90
119	16.96	28.80	37.60	33.63	33.60
120	18.58	25.80	40.80	35.74	33.50
121	21.99	30.80	38.40	34.56	33.60
122	13.96	25.60	29.60	26.94	26.10
123	13.01	24.00	33.20	29.36	25.60
124	8.21	25.00	55.20	49.97	26.60
128	17.16	27.60	35.20	31.57	30.10
129	24.43	31.20	35.60	31.30	29.60
130	25.89	33.20	39.20	34.46	30.40
131	16.18	28.60	32.80	29.06	26.60
132	21.49	30.20	36.40	31.86	28.10
133	13.19	25.40	30.80	27.75	25.20
201	11.73	30.80	47.20	41.27	30.70
202	14.37	35.00	39.60	35.34	33.50
203	16.54	27.60	32.00	27.58	26.80
204	10.85	31.40	34.80	30.54	28.40
205	10.64	25.60	32.00	29.03	26.30
206	16.23	26.80	31.60	28.68	26.00
207	14.10	17.60	19.60	17.93	15.80
208	12.07	17.60	20.00	18.20	15.40
209	12.19	15.00	20.00	18.09	15.90
210	9.41	14.80	16.40	14.83	13.50
211	9.42	13.60	16.00	14.59	12.60

Method Sample #	I	II	III	IV	V
212	5.96	15.60	17.60	15.61	13.40
213	15.37	25.00	34.40	30.95	28.30
214	17.35	28.40	33.60	30.62	37.40
215	15.79	27.60	32.80	28.92	33.70
216	18.55	28.00	29.60	26.85	45.20
217	12.93	24.80	28.40	25.69	26.00
218	13.07	26.00	28.40	25.12	26.60
219	12.75	21.20	24.00	21.48	24.80
220	24.25	23.20	27.20	24.62	25.10
221	22.54	25.20	28.80	25.31	23.30
222	15.72	21.40	22.80	20.16	19.90
223	13.78	20.40	24.40	22.18	19.00
224	14.04	21.80	26.40	23.89	20.60
301	21.37	28.80	32.40	29.47	27.00
302	15.14	27.00	34.00	31.63	26.40
303	14.66	24.80	32.00	31.17	24.80
304	24.86	26.80	28.80	26.78	22.60
305	22.98	28.20	32.40	30.71	25.30
306	8.23	13.60	17.20	16.51	12.40
307	13.83	17.20	20.80	20.21	15.70
308	7.88	14.80	18.00	17.32	13.40
309	8.70	17.60	22.80	20.85	17.20
310	7.24	15.00	18.00	16.22	13.00
311	9.13	14.60	18.40	16.08	15.50
312	10.04	29.20	27.20	25.33	23.00
314	13.00	21.80	29.20	27.07	23.50
315	10.99	21.00	26.80	23.64	20.80
317	17.15	22.80	26.80	21.56	22.50
318	13.68	22.20	26.80	19.51	19.70
319	10.67	17.20	22.40	19.29	19.20
320	12.50	18.80	27.20	19.73	19.60
321	15.96	22.60	28.00	25.07	22.20
322	11.04	17.80	22.00	19.89	18.20
323	14.62	22.40	24.80	22.88	20.90
324	18.53	24.80	35.20	32.12	23.20
401	17.38	24.00	26.00	23.83	21.60
402	16.69	24.20	24.40	21.92	21.90
403	13.53	22.60	24.80	22.07	20.60
404	15.96	24.20	25.60	22.98	21.10
405	12.53	20.60	24.00	21.14	17.80

Method Sample #	I	II	III	IV	V
406	10.93	22.40	25.60	22.59	20.50
407	10.26	15.20	18.00	15.65	14.20
408	7.53	13.20	14.00	12.49	10.60
409	11.75	15.80	17.20	15.57	13.70
410	8.12	13.20	13.60	12.11	10.10
411	8.97	13.60	13.60	12.41	10.30
412	9.29	14.20	15.60	14.35	10.80
413	11.35	18.80	22.00	19.63	17.30
414	11.69	23.00	24.80	22.26	18.60
415	11.98	18.40	23.20	20.36	16.90
416	12.54	21.40	21.60	19.20	16.50
417	9.51	20.40	22.00	19.61	16.20
418	12.11	20.80	23.60	20.71	16.40
419	8.69	15.80	20.80	17.98	14.60
420	12.63	19.40	21.20	18.82	15.40
421	14.62	21.20	21.60	19.49	13.70
422	12.07	18.20	19.20	17.36	15.10
423	12.10	19.40	20.80	18.42	16.20
424	14.41	20.80	20.40	18.27	17.40
501	12.37	19.80	24.40	21.96	14.30
502	10.74	17.20	19.60	17.57	13.80
503	11.89	17.00	70.80	62.19	12.10
504	9.10	15.00	66.00	59.97	21.20
505	11.79	19.60	71.60	64.79	16.30
506	8.78	17.80	71.20	63.59	9.90
507	6.93	11.60	65.20	59.10	7.90
508	5.15	9.40	59.60	53.47	12.80
509	6.17	12.20	15.60	14.52	12.90
510	6.01	10.20	14.40	13.48	9.70
511	5.90	9.60	12.00	11.07	8.40
512	5.85	9.80	14.40	13.46	10.40
513	8.12	13.20	18.40	16.75	13.90
514	7.30	14.20	19.20	17.17	14.30
515	6.99	13.00	15.20	13.83	11.30
516	8.37	15.00	17.20	15.78	12.80
517	10.29	14.80	16.80	15.29	13.10
518	6.08	12.00	15.60	14.29	10.90
519	7.72	14.40	16.40	14.67	13.10
520	5.41	11.20	21.60	19.38	12.90
521	10.24	18.40	19.60	18.13	15.10

Method / Sample #	I	II	III	IV	V
522	11.30	18.80	20.80	19.05	16.40
523	7.70	15.40	17.60	15.78	13.10
524	7.44	13.80	19.20	16.72	15.10
601	9.10	18.20	19.60	17.51	15.40
602	9.62	18.00	22.00	19.75	18.20
603	10.68	18.60	22.00	20.05	16.80
604	11.11	17.20	16.40	14.53	14.60
605	8.59	17.20	19.20	17.54	15.00
606	7.74	16.20	20.40	18.23	15.60
610	3.25	8.60	13.20	12.37	7.80
611	5.20	11.00	13.60	12.56	8.80
612	5.02	10.00	10.40	9.60	6.50
613	4.65	8.40	10.40	9.98	7.20
614	3.31	7.40	8.80	8.09	5.30
615	3.35	9.40	13.60	12.91	9.20
616	4.98	10.60	11.60	10.67	8.40
617	3.28	8.40	13.20	12.28	7.10
618	4.51	6.20	16.40	15.24	8.10
619	10.37	13.60	18.80	16.66	9.90
620	7.54	11.20	14.80	13.09	8.30
621	9.19	12.20	13.20	11.62	8.40
625	7.24	10.40	15.20	13.25	9.40
626	3.83	8.20	17.60	15.64	11.60
627	7.46	14.20	20.80	18.31	14.80
628	11.14	14.80	21.60	19.24	11.10
629	7.79	13.60	16.00	14.46	11.50
630	7.47	12.40	13.20	11.62	8.30

Appendix. 17. Maximum and minimum air temperature and precipitation in 1995-96, 1996-97 and 1997-98 growing seasons.

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	10/01/95	20.56	6.11	0.00
1995-96	10/02/95	19.44	7.78	0.00
1995-96	10/03/95	22.22	10.56	0.40
1995-96	10/04/95	18.89	6.67	0.02
1995-96	10/05/95	18.89	5.56	0.00
1995-96	10/06/95	21.11	6.67	0.02
1995-96	10/07/95	18.33	3.89	0.03
1995-96	10/08/95	19.44	6.11	0.00
1995-96	10/09/95	15.56	9.44	0.03
1995-96	10/10/95	20.00	9.44	0.00
1995-96	10/11/95	18.33	11.11	3.00
1995-96	10/12/95	16.11	6.11	0.22
1995-96	10/13/95	17.22	3.89	0.00
1995-96	10/14/95	17.78	4.44	0.00
1995-96	10/15/95	21.67	6.67	0.00
1995-96	10/16/95	23.33	7.22	0.14
1995-96	10/17/95	23.33	7.22	0.03
1995-96	10/18/95	15.00	2.22	0.39
1995-96	10/19/95	15.56	2.22	0.00
1995-96	10/20/95	21.11	3.33	0.00
1995-96	10/21/95	17.22	6.67	0.33
1995-96	10/22/95	15.56	1.67	0.02
1995-96	10/23/95	11.11	3.33	0.00
1995-96	10/24/95	13.89	6.11	0.09
1995-96	10/25/95	13.33	8.33	0.00
1995-96	10/26/95	12.78	8.89	1.23
1995-96	10/27/95	17.78	6.11	0.00
1995-96	10/28/95	16.11	6.11	0.00
1995-96	10/29/95	15.00	4.44	0.00
1995-96	10/30/95	13.89	3.89	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	10/31/95	12.22	-1.67	0.00
1995-96	11/01/95	11.67	2.78	0.00
1995-96	11/02/95	13.33	0.56	0.00
1995-96	11/03/95	11.67	-2.22	0.00
1995-96	11/04/95	12.22	-2.22	0.00
1995-96	11/05/95	10.56	0.56	0.06
1995-96	11/06/95	11.67	1.67	0.29
1995-96	11/07/95	12.22	7.78	0.70
1995-96	11/08/95	15.56	12.22	0.66
1995-96	11/09/95	16.11	6.67	0.51
1995-96	11/10/95	10.00	6.11	0.27
1995-96	11/11/95	13.89	7.22	0.63
1995-96	11/12/95	14.44	7.78	1.02
1995-96	11/13/95	14.44	8.33	0.68
1995-96	11/14/95	20.56	11.67	0.00
1995-96	11/15/95	15.56	12.22	0.00
1995-96	11/16/95	15.00	11.11	0.16
1995-96	11/17/95	15.00	10.56	0.00
1995-96	11/18/95	15.56	5.56	0.22
1995-96	11/19/95	15.56	6.67	0.00
1995-96	11/20/95	13.33	1.67	0.00
1995-96	11/21/95	11.67	1.67	0.00
1995-96	11/22/95	12.22	5.56	0.16
1995-96	11/23/95	15.00	6.11	0.21
1995-96	11/24/95	13.89	11.11	0.55
1995-96	11/25/95	17.22	10.00	0.99
1995-96	11/26/95	11.11	5.00	0.60
1995-96	11/27/95	11.11	7.22	0.22
1995-96	11/28/95	13.33	7.78	1.69
1995-96	11/29/95	14.44	11.67	0.14
1995-96	11/30/95	13.33	11.11	1.80
1995-96	12/01/95	12.78	7.78	1.35
1995-96	12/02/95	12.22	6.67	0.43
1995-96	12/03/95	11.11	2.78	0.08

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	12/04/95	11.67	3.89	0.38
1995-96	12/05/95	11.67	1.67	0.90
1995-96	12/06/95	6.67	1.67	0.09
1995-96	12/07/95	5.00	2.22	0.00
1995-96	12/08/95	8.89	1.67	0.33
1995-96	12/09/95	2.78	-1.11	0.69
1995-96	12/10/95	3.33	0.00	0.12
1995-96	12/11/95	13.33	2.78	2.25
1995-96	12/12/95	13.33	8.33	2.13
1995-96	12/13/95	14.44	7.22	1.14
1995-96	12/14/95	8.89	6.67	0.80
1995-96	12/15/95	10.00	2.22	1.32
1995-96	12/16/95	8.89	2.22	0.04
1995-96	12/17/95	8.89	4.44	0.03
1995-96	12/18/95	7.22	4.44	0.64
1995-96	12/19/95	7.22	4.44	0.09
1995-96	12/20/95	8.89	5.56	0.12
1995-96	12/21/95	9.44	2.78	0.14
1995-96	12/22/95	7.78	-2.22	0.00
1995-96	12/23/95	8.89	-3.33	0.00
1995-96	12/24/95	7.22	-4.44	0.00
1995-96	12/25/95	6.11	-4.44	0.00
1995-96	12/26/95	5.00	-5.00	0.00
1995-96	12/27/95	3.33	-3.33	0.02
1995-96	12/28/95	4.44	-0.56	0.16
1995-96	12/29/95	10.00	0.56	1.40
1995-96	12/30/95	12.78	9.44	0.22
1995-96	12/31/95	13.89	6.11	0.45
1995-96	01/01/96	12.22	3.89	0.00
1995-96	01/02/96	10.56	5.00	0.00
1995-96	01/03/96	13.89	7.22	0.16
1995-96	01/04/96	11.11	2.78	0.06
1995-96	01/05/96	8.89	3.89	0.11
1995-96	01/06/96	9.44	5.56	0.58

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	01/07/96	12.78	8.89	0.15
1995-96	01/08/96	11.11	8.33	0.76
1995-96	01/09/96	15.56	8.33	0.52
1995-96	01/10/96	11.11	4.44	0.24
1995-96	01/11/96	11.11	2.78	0.00
1995-96	01/12/96	5.56	2.78	0.03
1995-96	01/13/96	5.56	3.33	0.02
1995-96	01/14/96	8.89	4.44	0.08
1995-96	01/15/96	11.67	6.67	1.42
1995-96	01/16/96	12.22	6.11	1.41
1995-96	01/17/96	6.67	1.11	0.08
1995-96	01/18/96	7.78	0.00	0.03
1995-96	01/19/96	8.89	1.11	1.86
1995-96	01/20/96	9.44	5.00	0.70
1995-96	01/21/96	7.78	3.89	2.19
1995-96	01/22/96	7.22	0.00	0.29
1995-96	01/23/96	5.56	0.56	0.38
1995-96	01/24/96	6.11	1.67	1.83
1995-96	01/25/96	5.56	1.11	0.63
1995-96	01/26/96	7.22	0.56	0.12
1995-96	01/27/96	6.11	0.56	0.94
1995-96	01/28/96	4.44	0.56	0.68
1995-96	01/29/96	5.56	1.11	0.24
1995-96	01/30/96	5.56	-7.22	0.04
1995-96	01/31/96	-0.56	-7.22	0.00
1995-96	02/01/96	1.11	-7.22	0.00
1995-96	02/02/96	1.67	-7.78	0.00
1995-96	02/03/96	1.67	-7.22	0.00
1995-96	02/04/96	0.00	-5.56	0.38
1995-96	02/05/96	2.22	-1.67	0.38
1995-96	02/06/96	12.22	1.11	4.89
1995-96	02/07/96	13.89	11.11	3.58
1995-96	02/08/96	15.56	11.67	1.65
1995-96	02/09/96	16.67	5.00	1.96

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	02/10/96	10.00	5.00	0.00
1995-96	02/11/96	13.33	3.33	0.00
1995-96	02/12/96	13.89	0.00	0.00
1995-96	02/13/96	14.44	0.00	0.00
1995-96	02/14/96	16.67	0.00	0.00
1995-96	02/15/96	17.22	0.56	0.00
1995-96	02/16/96	16.67	1.67	0.06
1995-96	02/17/96	13.33	6.67	0.57
1995-96	02/18/96	14.44	8.89	1.56
1995-96	02/19/96	13.89	6.11	1.95
1995-96	02/20/96	12.78	5.56	0.42
1995-96	02/21/96	12.78	2.78	0.18
1995-96	02/22/96	7.22	1.11	0.51
1995-96	02/23/96	7.78	1.67	1.53
1995-96	02/24/96	6.67	0.00	0.76
1995-96	02/25/96	6.67	0.00	0.02
1995-96	02/26/96	3.89	-3.33	0.00
1995-96	02/27/96	4.44	-1.11	0.00
1995-96	02/28/96	6.67	-1.11	0.04
1995-96	02/29/96	10.56	0.00	0.00
1995-96	03/01/96	12.78	-2.22	0.00
1995-96	03/02/96	15.00	-1.67	0.00
1995-96	03/03/96	11.67	0.56	0.08
1995-96	03/04/96	14.44	7.22	1.22
1995-96	03/05/96	8.89	5.56	1.05
1995-96	03/06/96	11.11	4.44	0.08
1995-96	03/07/96	15.00	7.78	0.00
1995-96	03/08/96	12.22	8.33	0.18
1995-96	03/09/96	15.56	8.89	0.09
1995-96	03/10/96	15.00	9.44	1.36
1995-96	03/11/96	16.67	6.67	0.39
1995-96	03/12/96	12.22	5.00	0.12
1995-96	03/13/96	12.22	6.11	0.04
1995-96	03/14/96	15.56	3.33	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	03/15/96	19.44	4.44	0.00
1995-96	03/16/96	14.44	0.56	0.00
1995-96	03/17/96	15.00	5.00	0.00
1995-96	03/18/96	15.56	3.89	0.00
1995-96	03/19/96	20.56	8.33	1.35
1995-96	03/20/96	13.89	0.56	0.00
1995-96	03/21/96	15.00	1.11	0.00
1995-96	03/22/96	12.78	0.00	0.30
1995-96	03/23/96	10.00	2.78	0.12
1995-96	03/24/96	12.22	2.78	0.08
1995-96	03/25/96	11.11	-1.67	0.00
1995-96	03/26/96	12.22	-2.22	0.00
1995-96	03/27/96	16.67	1.67	0.00
1995-96	03/28/96	9.44	1.11	0.81
1995-96	03/29/96	14.44	3.89	0.04
1995-96	03/30/96	11.11	1.11	0.21
1995-96	03/31/96	12.22	6.11	0.51
1995-96	04/01/96	12.78	7.78	0.69
1995-96	04/02/96	16.11	6.67	0.14
1995-96	04/03/96	12.78	1.67	0.06
1995-96	04/04/96	16.67	2.22	0.00
1995-96	04/05/96	21.67	6.11	0.00
1995-96	04/06/96	23.89	6.67	0.00
1995-96	04/07/96	25.56	9.44	0.00
1995-96	04/08/96	26.11	7.78	0.00
1995-96	04/09/96	21.67	9.44	0.14
1995-96	04/10/96	17.22	6.67	0.03
1995-96	04/11/96	12.22	7.78	0.03
1995-96	04/12/96	11.11	5.00	1.11
1995-96	04/13/96	11.11	2.78	0.26
1995-96	04/14/96	16.67	5.00	0.00
1995-96	04/15/96	21.67	7.78	0.00
1995-96	04/16/96	18.33	6.67	0.29
1995-96	04/17/96	12.78	3.89	0.18

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	04/18/96	11.67	2.78	0.15
1995-96	04/19/96	11.67	4.44	0.21
1995-96	04/20/96	11.11	5.00	0.45
1995-96	04/21/96	12.78	5.56	0.40
1995-96	04/22/96	14.44	6.67	0.69
1995-96	04/23/96	13.89	9.44	1.05
1995-96	04/24/96	15.56	6.67	1.64
1995-96	04/25/96	16.11	7.78	0.04
1995-96	04/26/96	16.67	2.22	0.08
1995-96	04/27/96	16.11	3.33	0.04
1995-96	04/28/96	15.56	4.44	0.00
1995-96	04/29/96	19.44	6.11	0.00
1995-96	04/30/96	21.67	6.67	0.00
1995-96	05/01/96	22.22	7.78	0.02
1995-96	05/02/96	17.78	7.22	0.00
1995-96	05/03/96	14.44	0.56	0.03
1995-96	05/04/96	11.67	-0.56	0.16
1995-96	05/05/96	15.56	0.00	0.00
1995-96	05/06/96	18.33	3.89	0.00
1995-96	05/07/96	18.89	2.22	0.14
1995-96	05/08/96	14.44	-1.11	0.09
1995-96	05/09/96	15.56	1.11	0.00
1995-96	05/10/96	16.11	5.00	0.00
1995-96	05/11/96	16.67	7.22	0.00
1995-96	05/12/96	20.00	11.11	0.02
1995-96	05/13/96	19.44	13.33	0.60
1995-96	05/14/96	18.89	12.22	0.39
1995-96	05/15/96	18.89	11.11	0.36
1995-96	05/16/96	18.89	7.78	0.15
1995-96	05/17/96	19.44	10.56	0.93
1995-96	05/18/96	16.67	10.00	0.84
1995-96	05/19/96	15.56	6.67	1.10
1995-96	05/20/96	16.67	3.89	0.04
1995-96	05/21/96	18.33	6.11	0.45

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	05/22/96	12.78	6.67	0.40
1995-96	05/23/96	15.00	7.22	0.21
1995-96	05/24/96	16.11	6.67	0.02
1995-96	05/25/96	21.67	7.78	0.00
1995-96	05/26/96	26.11	7.78	0.00
1995-96	05/27/96	20.00	3.33	0.00
1995-96	05/28/96	16.11	4.44	0.00
1995-96	05/29/96	16.11	7.78	0.09
1995-96	05/30/96	16.67	8.33	0.08
1995-96	05/31/96	17.78	4.44	0.00
1995-96	06/01/96	22.22	10.56	0.00
1995-96	06/02/96	26.67	11.11	0.00
1995-96	06/03/96	30.00	12.22	0.00
1995-96	06/04/96	26.67	8.33	0.00
1995-96	06/05/96	21.11	9.44	0.00
1995-96	06/06/96	24.44	7.78	0.00
1995-96	06/07/96	28.89	9.44	0.00
1995-96	06/08/96	22.78	8.33	0.00
1995-96	06/09/96	22.22	6.11	0.00
1995-96	06/10/96	19.44	5.00	0.00
1995-96	06/11/96	22.78	8.33	0.00
1995-96	06/12/96	21.67	7.78	0.00
1995-96	06/13/96	25.56	7.22	0.00
1995-96	06/14/96	22.78	6.11	0.00
1995-96	06/15/96	23.89	6.11	0.00
1995-96	06/16/96	22.78	6.11	0.00
1995-96	06/17/96	19.44	3.33	0.00
1995-96	06/18/96	17.22	6.67	0.14
1995-96	06/19/96	20.00	9.44	0.02
1995-96	06/20/96	26.11	7.78	0.00
1995-96	06/21/96	24.44	5.56	0.00
1995-96	06/22/96	21.11	6.67	0.00
1995-96	06/23/96	23.89	11.11	0.57
1995-96	06/24/96	19.44	11.67	0.42

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	06/25/96	20.00	11.11	0.02
1995-96	06/26/96	22.78	7.22	0.00
1995-96	06/27/96	26.67	12.22	0.14
1995-96	06/28/96	20.56	12.78	0.12
1995-96	06/29/96	21.67	7.78	0.00
1995-96	06/30/96	22.22	8.33	0.00
1995-96	07/01/96	27.78	10.00	0.00
1995-96	07/02/96	30.00	12.22	0.00
1995-96	07/03/96	28.89	12.22	0.00
1995-96	07/04/96	26.11	11.11	0.00
1995-96	07/05/96	22.78	8.89	0.00
1995-96	07/06/96	25.00	10.00	0.00
1995-96	07/07/96	29.44	12.78	0.00
1995-96	07/08/96	33.89	13.33	0.00
1995-96	07/09/96	30.56	12.22	0.00
1995-96	07/10/96	23.89	8.89	0.00
1995-96	07/11/96	27.78	8.89	0.00
1995-96	07/12/96	32.22	12.78	0.00
1995-96	07/13/96	35.56	14.44	0.00
1995-96	07/14/96	37.22	15.56	0.00
1995-96	07/15/96	37.22	12.22	0.00
1995-96	07/16/96	29.44	7.22	0.00
1995-96	07/17/96	26.67	12.78	0.30
1995-96	07/18/96	16.67	10.56	1.01
1995-96	07/19/96	20.00	10.56	0.08
1995-96	07/20/96	23.89	9.44	0.00
1995-96	07/21/96	25.56	14.44	0.00
1995-96	07/22/96	27.22	14.44	0.00
1995-96	07/23/96	34.44	16.67	0.00
1995-96	07/24/96	36.11	15.00	0.00
1995-96	07/25/96	35.00	13.33	0.00
1995-96	07/26/96	35.00	13.89	0.00
1995-96	07/27/96	36.11	15.00	0.00
1995-96	07/28/96	33.33	14.44	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1995-96	07/29/96	28.33	14.44	0.14
1995-96	07/30/96	32.78	13.89	0.00
1995-96	07/31/96	30.00	10.00	0.00
1995-96	08/01/96	28.89	8.89	0.00
1995-96	08/02/96	26.11	11.67	0.21
1995-96	08/03/96	21.67	11.11	0.14
1995-96	08/04/96	24.44	10.00	0.00
1995-96	08/05/96	22.78	11.67	0.00
1996-97	10/01/96	23.33	10.56	0.00
1996-97	10/02/96	22.22	6.11	0.00
1996-97	10/03/96	23.33	9.44	0.00
1996-97	10/04/96	25.56	10.00	0.00
1996-97	10/05/96	23.89	12.22	0.58
1996-97	10/06/96	18.33	6.11	0.00
1996-97	10/07/96	21.67	8.33	0.00
1996-97	10/08/96	27.22	9.44	0.00
1996-97	10/09/96	25.00	9.44	0.00
1996-97	10/10/96	27.78	11.11	0.00
1996-97	10/11/96	18.89	11.11	0.00
1996-97	10/12/96	18.89	6.11	0.00
1996-97	10/13/96	16.11	8.89	0.96
1996-97	10/14/96	16.67	5.56	0.20
1996-97	10/15/96	13.33	6.67	0.48
1996-97	10/16/96	15.00	3.89	0.42
1996-97	10/17/96	10.56	1.67	0.00
1996-97	10/18/96	13.33	2.78	0.69
1996-97	10/19/96	10.56	3.33	0.99
1996-97	10/20/96	12.22	3.89	0.09
1996-97	10/21/96	13.33	1.11	0.00
1996-97	10/22/96	12.78	3.33	0.34
1996-97	10/23/96	13.89	4.44	0.00
1996-97	10/24/96	13.33	6.11	1.14
1996-97	10/25/96	15.56	6.67	1.30
1996-97	10/26/96	13.33	0.00	0.08

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	10/27/96	10.56	0.56	0.00
1996-97	10/28/96	13.33	3.33	0.00
1996-97	10/29/96	10.00	3.33	0.70
1996-97	10/30/96	11.11	1.67	0.00
1996-97	10/31/96	16.11	5.00	0.00
1996-97	11/01/96	14.44	-0.56	0.00
1996-97	11/02/96	14.44	1.11	0.00
1996-97	11/03/96	11.11	2.78	0.00
1996-97	11/04/96	14.44	3.89	0.15
1996-97	11/05/96	11.67	1.67	0.15
1996-97	11/06/96	11.67	3.33	0.00
1996-97	11/07/96	12.22	4.44	0.38
1996-97	11/08/96	12.78	3.89	0.00
1996-97	11/09/96	14.44	3.89	0.00
1996-97	11/10/96	8.33	4.44	0.00
1996-97	11/11/96	8.33	4.44	0.00
1996-97	11/12/96	12.78	5.00	0.22
1996-97	11/13/96	16.67	5.00	0.43
1996-97	11/14/96	13.89	6.67	0.20
1996-97	11/15/96	13.89	5.00	0.15
1996-97	11/16/96	11.11	6.11	0.38
1996-97	11/17/96	10.00	6.67	0.93
1996-97	11/18/96	10.56	5.56	0.94
1996-97	11/19/96	7.78	1.11	6.68
1996-97	11/20/96	14.44	0.56	0.75
1996-97	11/21/96	10.56	2.78	0.04
1996-97	11/22/96	5.00	3.33	1.20
1996-97	11/23/96	6.67	-0.56	0.00
1996-97	11/24/96	10.00	1.67	0.76
1996-97	11/25/96	15.00	2.78	0.38
1996-97	11/26/96	10.00	3.89	0.03
1996-97	11/27/96	10.00	4.44	0.43
1996-97	11/28/96	12.78	7.22	0.88
1996-97	11/29/96	11.11	2.78	0.18

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	11/30/96	8.89	3.33	0.03
1996-97	12/01/96	12.22	5.00	1.05
1996-97	12/02/96	8.33	3.33	0.16
1996-97	12/03/96	6.67	-0.56	0.86
1996-97	12/04/96	8.89	1.11	0.14
1996-97	12/05/96	11.67	4.44	2.20
1996-97	12/06/96	8.33	3.33	1.69
1996-97	12/07/96	7.22	4.44	1.23
1996-97	12/08/96	10.56	6.11	2.20
1996-97	12/09/96	8.33	5.00	0.16
1996-97	12/10/96	9.44	4.44	0.64
1996-97	12/11/96	8.33	3.89	0.46
1996-97	12/12/96	6.11	3.33	1.12
1996-97	12/13/96	8.89	5.56	0.92
1996-97	12/14/96	10.56	0.00	0.12
1996-97	12/15/96	5.00	0.00	0.00
1996-97	12/16/96	5.56	1.67	0.00
1996-97	12/17/96	6.11	1.67	0.00
1996-97	12/18/96	7.78	-1.11	0.00
1996-97	12/19/96	5.00	-3.33	0.00
1996-97	12/20/96	7.78	-3.33	0.20
1996-97	12/21/96	9.44	2.78	0.74
1996-97	12/22/96	5.56	-0.56	0.38
1996-97	12/23/96	6.11	0.56	0.45
1996-97	12/24/96	8.33	4.44	1.08
1996-97	12/25/96	10.00	7.78	1.56
1996-97	12/26/96	11.11	1.67	2.25
1996-97	12/27/96	11.67	2.22	1.54
1996-97	12/28/96	7.78	-1.67	0.00
1996-97	12/29/96	11.67	-0.56	1.92
1996-97	12/30/96	12.78	5.56	0.94
1996-97	12/31/96	11.67	8.33	1.64
1996-97	01/01/97	15.00	8.33	1.96
1996-97	01/02/97	12.22	10.00	2.19

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	01/03/97	13.89	3.89	0.54
1996-97	01/04/97	9.44	0.00	0.04
1996-97	01/05/97	5.00	0.56	0.09
1996-97	01/06/97	5.00	1.67	0.00
1996-97	01/07/97	6.11	3.33	0.06
1996-97	01/08/97	8.89	2.22	0.00
1996-97	01/09/97	7.78	4.44	0.02
1996-97	01/10/97	10.00	4.44	0.03
1996-97	01/11/97	14.44	4.44	0.00
1996-97	01/12/97	8.89	-1.11	0.00
1996-97	01/13/97	5.00	-2.78	0.00
1996-97	01/14/97	4.44	-6.67	0.00
1996-97	01/15/97	5.00	-6.67	0.00
1996-97	01/16/97	5.00	-4.44	0.00
1996-97	01/17/97	3.33	0.00	0.57
1996-97	01/18/97	10.00	2.78	2.80
1996-97	01/19/97	10.56	5.56	0.02
1996-97	01/20/97	10.00	6.11	0.54
1996-97	01/21/97	10.56	5.00	0.45
1996-97	01/22/97	7.78	0.56	0.08
1996-97	01/23/97	10.00	-1.11	0.04
1996-97	01/24/97	7.78	0.00	0.08
1996-97	01/25/97	5.00	0.00	0.00
1996-97	01/26/97	3.89	-1.11	0.50
1996-97	01/27/97	5.00	-1.11	0.00
1996-97	01/28/97	5.00	0.00	0.70
1996-97	01/29/97	13.89	3.33	0.00
1996-97	01/30/97	11.67	5.00	0.16
1996-97	01/31/97	12.22	10.00	2.74
1996-97	02/01/97	13.33	6.11	0.86
1996-97	02/02/97	10.56	1.67	0.09
1996-97	02/03/97	8.33	1.67	0.00
1996-97	02/04/97	6.67	3.33	0.00
1996-97	02/05/97	10.56	-0.56	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	02/06/97	9.44	-3.33	0.00
1996-97	02/07/97	6.67	-2.22	0.27
1996-97	02/08/97	3.89	1.11	0.00
1996-97	02/09/97	6.67	0.56	0.00
1996-97	02/10/97	6.67	0.00	0.00
1996-97	02/11/97	10.00	-1.11	0.00
1996-97	02/12/97	7.78	1.67	0.57
1996-97	02/13/97	10.00	2.22	0.04
1996-97	02/14/97	9.44	4.44	0.00
1996-97	02/15/97	15.56	2.22	0.00
1996-97	02/16/97	13.33	3.33	0.00
1996-97	02/17/97	14.44	5.00	0.08
1996-97	02/18/97	12.78	4.44	0.00
1996-97	02/19/97	12.22	6.11	0.80
1996-97	02/20/97	11.11	1.67	0.26
1996-97	02/21/97	11.67	3.33	0.00
1996-97	02/22/97	11.11	0.00	0.00
1996-97	02/23/97	12.78	2.22	0.00
1996-97	02/24/97	15.00	3.89	0.00
1996-97	02/25/97	16.11	0.56	0.00
1996-97	02/26/97	11.11	2.78	0.18
1996-97	02/27/97	10.56	3.33	0.08
1996-97	02/28/97	10.00	1.11	0.09
1996-97	03/01/97	10.56	2.22	0.18
1996-97	03/02/97	8.89	3.33	2.22
1996-97	03/03/97	6.67	0.56	0.48
1996-97	03/04/97	7.22	0.56	0.21
1996-97	03/05/97	10.00	1.67	0.00
1996-97	03/06/97	7.78	4.44	0.51
1996-97	03/07/97	11.67	7.22	0.26
1996-97	03/08/97	11.67	-0.56	0.00
1996-97	03/09/97	11.11	3.89	0.24
1996-97	03/10/97	12.22	6.11	1.14
1996-97	03/11/97	7.22	3.33	0.93

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	03/12/97	10.56	2.22	0.30
1996-97	03/13/97	8.89	1.11	0.12
1996-97	03/14/97	11.11	2.78	0.00
1996-97	03/15/97	10.00	3.89	0.43
1996-97	03/16/97	14.44	8.33	0.18
1996-97	03/17/97	11.67	7.22	0.81
1996-97	03/18/97	16.11	7.78	0.00
1996-97	03/19/97	18.33	11.11	0.22
1996-97	03/20/97	16.11	6.67	0.62
1996-97	03/21/97	14.44	1.11	0.00
1996-97	03/22/97	16.11	2.78	0.00
1996-97	03/23/97	17.22	4.44	0.00
1996-97	03/24/97	15.56	3.89	0.00
1996-97	03/25/97	18.89	3.33	0.00
1996-97	03/26/97	21.67	7.78	0.06
1996-97	03/27/97	14.44	1.11	0.09
1996-97	03/28/97	12.78	1.11	0.20
1996-97	03/29/97	13.33	-0.56	0.00
1996-97	03/30/97	15.56	3.33	0.00
1996-97	03/31/97	13.33	1.11	0.45
1996-97	04/01/97	9.44	0.00	0.22
1996-97	04/02/97	12.22	0.56	0.00
1996-97	04/03/97	15.00	1.67	0.00
1996-97	04/04/97	13.89	-2.78	0.00
1996-97	04/05/97	12.78	1.11	0.00
1996-97	04/06/97	16.67	0.00	0.00
1996-97	04/07/97	17.22	5.00	0.15
1996-97	04/08/97	13.33	6.67	0.30
1996-97	04/09/97	13.33	-0.56	0.03
1996-97	04/10/97	13.33	1.11	0.04
1996-97	04/11/97	13.89	2.78	0.00
1996-97	04/12/97	16.67	1.67	0.00
1996-97	04/13/97	13.89	5.56	0.00
1996-97	04/14/97	12.22	8.33	0.39

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	04/15/97	14.44	7.22	0.34
1996-97	04/16/97	23.33	7.78	0.08
1996-97	04/17/97	18.89	2.78	0.00
1996-97	04/18/97	17.78	7.22	0.11
1996-97	04/19/97	13.89	8.89	0.11
1996-97	04/20/97	15.00	8.33	1.62
1996-97	04/21/97	17.22	6.11	0.00
1996-97	04/22/97	13.89	7.22	0.11
1996-97	04/23/97	14.44	6.67	0.99
1996-97	04/24/97	15.00	5.56	0.11
1996-97	04/25/97	15.56	3.33	0.00
1996-97	04/26/97	22.22	7.22	0.00
1996-97	04/27/97	23.33	5.00	0.15
1996-97	04/28/97	14.44	7.78	0.12
1996-97	04/29/97	13.33	5.56	0.20
1996-97	04/30/97	16.11	8.33	0.34
1996-97	05/01/97	13.33	2.22	0.08
1996-97	05/02/97	15.00	3.89	0.02
1996-97	05/03/97	18.89	5.56	0.12
1996-97	05/04/97	14.44	8.89	0.03
1996-97	05/05/97	20.00	8.89	0.00
1996-97	05/06/97	21.11	7.78	0.14
1996-97	05/07/97	18.33	6.11	0.00
1996-97	05/08/97	21.67	5.00	0.00
1996-97	05/09/97	25.56	6.11	0.00
1996-97	05/10/97	25.00	9.44	0.00
1996-97	05/11/97	27.22	12.78	0.00
1996-97	05/12/97	31.67	10.56	0.00
1996-97	05/13/97	28.33	6.11	0.00
1996-97	05/14/97	27.78	9.44	0.00
1996-97	05/15/97	26.67	14.44	0.00
1996-97	05/16/97	25.56	10.56	0.00
1996-97	05/17/97	27.22	12.78	0.00
1996-97	05/18/97	27.22	7.78	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	05/19/97	27.22	7.22	0.00
1996-97	05/20/97	26.67	2.78	0.00
1996-97	05/21/97	21.11	4.44	0.00
1996-97	05/22/97	21.67	3.33	0.00
1996-97	05/23/97	20.00	9.44	0.32
1996-97	05/24/97	18.33	5.56	0.24
1996-97	05/25/97	17.22	3.89	0.09
1996-97	05/26/97	18.33	3.89	0.02
1996-97	05/27/97	23.33	11.11	0.03
1996-97	05/28/97	24.44	11.11	0.11
1996-97	05/29/97	21.11	15.56	1.12
1996-97	05/30/97	26.11	16.11	0.12
1996-97	05/31/97	26.11	16.11	0.88
1996-97	06/01/97	19.44	11.11	0.96
1996-97	06/02/97	20.56	5.56	0.00
1996-97	06/03/97	23.33	11.67	0.22
1996-97	06/04/97	19.44	11.67	0.26
1996-97	06/05/97	19.44	6.11	0.20
1996-97	06/06/97	21.11	5.00	0.00
1996-97	06/07/97	23.33	11.67	0.00
1996-97	06/08/97	23.89	7.22	0.00
1996-97	06/09/97	21.67	7.78	0.00
1996-97	06/10/97	26.67	7.78	0.00
1996-97	06/11/97	26.67	9.44	0.00
1996-97	06/12/97	18.33	9.44	0.26
1996-97	06/13/97	19.44	6.67	0.60
1996-97	06/14/97	23.89	8.33	0.00
1996-97	06/15/97	24.44	10.00	0.00
1996-97	06/16/97	28.33	10.00	0.00
1996-97	06/17/97	28.33	12.78	0.00
1996-97	06/18/97	23.89	13.33	0.03
1996-97	06/19/97	22.22	7.22	0.00
1996-97	06/20/97	22.22	5.56	0.00
1996-97	06/21/97	24.44	11.11	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	06/22/97	17.78	7.22	0.40
1996-97	06/23/97	18.33	10.56	0.42
1996-97	06/24/97	19.44	6.67	0.04
1996-97	06/25/97	22.78	7.78	0.00
1996-97	06/26/97	22.78	6.67	0.00
1996-97	06/27/97	22.22	7.78	0.00
1996-97	06/28/97	22.78	10.56	0.00
1996-97	06/29/97	21.11	6.67	0.24
1996-97	06/30/97	23.33	10.56	0.00
1996-97	07/01/97	21.11	10.56	0.20
1996-97	07/02/97	22.78	8.33	0.00
1996-97	07/03/97	25.56	12.22	0.00
1996-97	07/04/97	30.00	12.22	0.00
1996-97	07/05/97	32.22	14.44	0.00
1996-97	07/06/97	26.67	14.44	0.02
1996-97	07/07/97	25.56	8.33	0.11
1996-97	07/08/97	27.22	13.33	0.00
1996-97	07/09/97	24.44	13.33	0.26
1996-97	07/10/97	20.56	11.67	0.00
1996-97	07/11/97	21.11	8.33	0.00
1996-97	07/12/97	23.33	11.11	0.00
1996-97	07/13/97	25.00	12.22	0.00
1996-97	07/14/97	28.33	13.89	0.00
1996-97	07/15/97	28.89	10.56	0.00
1996-97	07/16/97	26.67	13.89	0.00
1996-97	07/17/97	28.33	12.22	0.00
1996-97	07/18/97	22.22	9.44	0.00
1996-97	07/19/97	26.67	11.11	0.00
1996-97	07/20/97	31.11	12.22	0.00
1996-97	07/21/97	31.67	11.11	0.00
1996-97	07/22/97	25.00	10.00	0.00
1996-97	07/23/97	26.67	12.22	0.00
1996-97	07/24/97	27.78	10.00	0.00
1996-97	07/25/97	28.89	10.56	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1996-97	07/26/97	27.22	10.00	0.00
1996-97	07/27/97	28.33	11.67	0.00
1996-97	07/28/97	31.67	11.67	0.00
1996-97	07/29/97	30.56	10.56	0.00
1996-97	07/30/97	26.67	12.22	0.00
1996-97	07/31/97	27.22	8.89	0.00
1996-97	08/01/97	28.89	8.33	0.00
1996-97	08/02/97	30.00	9.44	0.00
1996-97	08/03/97	28.89	11.11	0.00
1996-97	08/04/97	30.00	10.56	0.00
1996-97	08/05/97	31.11	13.33	0.00
1997-98	10/01/97	18.33	13.89	0.20
1997-98	10/02/97	16.11	11.11	1.20
1997-98	10/03/97	19.44	11.67	0.16
1997-98	10/04/97	17.78	12.22	0.69
1997-98	10/05/97	16.67	7.22	0.18
1997-98	10/06/97	17.22	7.78	0.16
1997-98	10/07/97	15.56	3.33	0.00
1997-98	10/08/97	15.56	3.89	0.14
1997-98	10/09/97	15.56	8.33	1.30
1997-98	10/10/97	12.22	6.67	0.62
1997-98	10/11/97	12.78	3.89	0.22
1997-98	10/12/97	15.00	7.22	0.04
1997-98	10/13/97	15.56	6.11	0.00
1997-98	10/14/97	15.56	3.89	0.00
1997-98	10/15/97	18.33	6.11	0.00
1997-98	10/16/97	20.56	8.33	0.00
1997-98	10/17/97	18.89	7.22	0.03
1997-98	10/18/97	16.11	8.33	0.02
1997-98	10/19/97	15.56	5.00	0.00
1997-98	10/20/97	15.56	0.56	0.00
1997-98	10/21/97	16.11	1.11	0.00
1997-98	10/22/97	14.44	3.33	0.00
1997-98	10/23/97	14.44	5.56	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	10/24/97	15.00	1.67	0.00
1997-98	10/25/97	13.33	0.56	0.00
1997-98	10/26/97	13.89	1.11	0.00
1997-98	10/27/97	12.22	5.00	0.22
1997-98	10/28/97	15.00	5.00	0.00
1997-98	10/29/97	13.33	9.44	1.29
1997-98	10/30/97	16.11	12.22	0.84
1997-98	10/31/97	17.22	7.78	0.93
1997-98	11/01/97	16.67	5.56	0.00
1997-98	11/02/97	16.11	2.78	0.00
1997-98	11/03/97	13.33	3.89	0.00
1997-98	11/04/97	17.78	8.33	0.03
1997-98	11/05/97	16.67	9.44	0.00
1997-98	11/06/97	18.33	11.67	0.12
1997-98	11/07/97	16.11	8.89	0.42
1997-98	11/08/97	13.33	1.67	0.04
1997-98	11/09/97	7.78	3.33	0.00
1997-98	11/10/97	11.67	2.78	0.00
1997-98	11/11/97	15.56	5.56	0.00
1997-98	11/12/97	12.78	7.22	0.00
1997-98	11/13/97	15.56	2.78	0.00
1997-98	11/14/97	10.56	2.22	0.00
1997-98	11/15/97	15.56	2.22	0.00
1997-98	11/16/97	12.78	2.22	0.03
1997-98	11/17/97	11.11	5.56	0.84
1997-98	11/18/97	12.78	2.78	0.36
1997-98	11/19/97	9.44	5.00	0.84
1997-98	11/20/97	10.56	5.00	1.84
1997-98	11/21/97	13.33	7.22	0.12
1997-98	11/22/97	13.89	6.67	0.04
1997-98	11/23/97	12.22	7.78	0.43
1997-98	11/24/97	13.33	3.33	1.36
1997-98	11/25/97	11.67	1.67	0.29
1997-98	11/26/97	12.78	1.67	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	11/27/97	11.67	0.00	0.00
1997-98	11/28/97	12.22	5.56	0.00
1997-98	11/29/97	13.89	7.22	0.42
1997-98	11/30/97	11.11	2.22	0.57
1997-98	12/01/97	11.11	2.78	0.00
1997-98	12/02/97	8.33	-1.67	0.00
1997-98	12/03/97	4.44	-2.22	0.00
1997-98	12/04/97	8.33	-1.67	0.00
1997-98	12/05/97	10.00	1.67	0.00
1997-98	12/06/97	8.89	-2.78	0.00
1997-98	12/07/97	6.11	-2.22	0.29
1997-98	12/08/97	6.67	0.00	0.32
1997-98	12/09/97	6.67	-0.56	0.39
1997-98	12/10/97	6.67	0.56	0.03
1997-98	12/11/97	11.11	-0.56	0.00
1997-98	12/12/97	3.33	-1.11	0.00
1997-98	12/13/97	3.89	-1.11	0.00
1997-98	12/14/97	3.89	-1.11	0.11
1997-98	12/15/97	8.89	-0.56	0.09
1997-98	12/16/97	10.56	2.78	0.75
1997-98	12/17/97	11.67	5.00	1.34
1997-98	12/18/97	11.11	-2.22	0.00
1997-98	12/19/97	2.78	-1.11	0.02
1997-98	12/20/97	7.22	-0.56	0.46
1997-98	12/21/97	9.44	-0.56	0.33
1997-98	12/22/97	7.78	-1.67	0.03
1997-98	12/23/97	2.78	-1.67	0.12
1997-98	12/24/97	6.11	2.78	0.24
1997-98	12/25/97	5.00	1.67	0.00
1997-98	12/26/97	3.33	1.11	0.00
1997-98	12/27/97	6.67	1.11	0.03
1997-98	12/28/97	8.89	5.00	0.00
1997-98	12/29/97	11.11	2.22	0.00
1997-98	12/30/97	7.78	2.78	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	12/31/97	6.67	2.22	0.00
1997-98	01/01/98	9.44	1.67	0.00
1997-98	01/02/98	10.00	-2.22	0.36
1997-98	01/03/98	7.22	-0.56	0.00
1997-98	01/04/98	5.56	0.00	0.62
1997-98	01/05/98	8.89	1.11	0.48
1997-98	01/06/98	7.78	5.00	0.46
1997-98	01/07/98	5.56	3.33	0.36
1997-98	01/08/98	10.00	-2.78	0.00
1997-98	01/09/98	5.56	-1.67	0.00
1997-98	01/10/98	4.44	0.00	0.14
1997-98	01/11/98	4.44	0.56	1.65
1997-98	01/12/98	10.56	-1.67	0.29
1997-98	01/13/98	7.78	-2.22	1.74
1997-98	01/14/98	11.11	6.67	0.55
1997-98	01/15/98	11.11	1.67	0.74
1997-98	01/16/98	11.67	2.22	0.78
1997-98	01/17/98	13.89	7.78	0.72
1997-98	01/18/98	12.78	7.22	0.22
1997-98	01/19/98	10.56	5.56	0.45
1997-98	01/20/98	8.33	2.22	0.32
1997-98	01/21/98	11.11	0.56	0.03
1997-98	01/22/98	8.33	4.44	0.32
1997-98	01/23/98	10.56	6.67	0.86
1997-98	01/24/98	11.11	6.67	0.62
1997-98	01/25/98	10.00	4.44	1.14
1997-98	01/26/98	11.11	5.00	0.33
1997-98	01/27/98	11.11	6.67	0.06
1997-98	01/28/98	12.78	3.89	0.00
1997-98	01/29/98	12.22	5.56	0.18
1997-98	01/30/98	13.33	3.33	0.18
1997-98	01/31/98	12.78	1.67	0.00
1997-98	02/01/98	13.33	2.22	0.06
1997-98	02/02/98	11.67	1.67	0.33

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	02/03/98	12.22	2.78	0.33
1997-98	02/04/98	10.56	3.33	0.12
1997-98	02/05/98	12.22	3.89	0.15
1997-98	02/06/98	11.67	6.11	0.64
1997-98	02/07/98	-17.78	1.67	0.00
1997-98	02/08/98	11.11	2.78	0.38
1997-98	02/09/98	8.89	0.00	0.24
1997-98	02/10/98	10.00	2.22	0.09
1997-98	02/11/98	10.00	5.56	0.99
1997-98	02/12/98	12.22	6.11	0.12
1997-98	02/13/98	12.22	4.44	1.68
1997-98	02/14/98	13.33	5.00	0.42
1997-98	02/15/98	10.00	4.44	0.26
1997-98	02/16/98	11.67	1.11	0.03
1997-98	02/17/98	11.67	3.33	0.04
1997-98	02/18/98	12.22	5.00	0.27
1997-98	02/19/98	13.33	5.56	0.42
1997-98	02/20/98	10.56	6.11	0.14
1997-98	02/21/98	8.33	3.89	1.95
1997-98	02/22/98	8.33	2.78	1.78
1997-98	02/23/98	10.56	0.00	0.06
1997-98	02/24/98	10.56	-1.11	0.00
1997-98	02/25/98	11.67	1.11	0.21
1997-98	02/26/98	10.56	1.67	0.08
1997-98	02/27/98	11.11	-1.11	0.00
1997-98	02/28/98	10.00	0.56	0.76
1997-98	03/01/98	12.22	8.33	0.62
1997-98	03/02/98	11.67	5.56	0.18
1997-98	03/03/98	9.44	2.22	0.64
1997-98	03/04/98	7.22	1.67	0.50
1997-98	03/05/98	10.56	-2.22	0.06
1997-98	03/06/98	12.22	-2.22	0.04
1997-98	03/07/98	12.22	-3.33	0.00
1997-98	03/08/98	10.00	-1.11	0.66

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	03/09/98	11.11	5.00	0.12
1997-98	03/10/98	12.22	0.00	0.00
1997-98	03/11/98	11.11	2.78	0.39
1997-98	03/12/98	21.11	7.78	0.02
1997-98	03/13/98	14.44	7.22	0.39
1997-98	03/14/98	17.78	7.22	0.00
1997-98	03/15/98	18.33	7.22	0.20
1997-98	03/16/98	14.44	3.89	0.00
1997-98	03/17/98	13.89	-0.56	0.00
1997-98	03/18/98	13.33	1.67	0.00
1997-98	03/19/98	16.67	1.11	0.00
1997-98	03/20/98	18.89	2.78	0.00
1997-98	03/21/98	21.11	5.56	0.18
1997-98	03/22/98	12.22	8.33	0.00
1997-98	03/23/98	17.22	10.00	0.72
1997-98	03/24/98	15.56	7.22	0.58
1997-98	03/25/98	15.56	8.33	0.60
1997-98	03/26/98	15.00	6.11	0.15
1997-98	03/27/98	12.22	2.78	0.11
1997-98	03/28/98	8.33	-17.78	0.42
1997-98	03/29/98	11.11	-1.11	0.00
1997-98	03/30/98	14.44	1.11	0.00
1997-98	03/31/98	10.00	5.00	0.36
1997-98	04/01/98	11.11	2.78	0.02
1997-98	04/02/98	12.78	4.44	0.00
1997-98	04/03/98	13.33	5.00	0.11
1997-98	04/04/98	10.56	2.78	0.08
1997-98	04/05/98	13.33	3.89	0.14
1997-98	04/06/98	14.44	4.44	0.18
1997-98	04/07/98	13.89	3.33	0.16
1997-98	04/08/98	13.33	0.00	0.09
1997-98	04/09/98	13.89	3.89	0.14
1997-98	04/10/98	13.89	4.44	0.68
1997-98	04/11/98	13.33	5.56	0.33

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	04/12/98	12.78	3.89	0.08
1997-98	04/13/98	11.11	-0.56	0.03
1997-98	04/14/98	13.89	2.78	0.00
1997-98	04/15/98	13.33	2.22	0.04
1997-98	04/16/98	14.44	0.00	0.00
1997-98	04/17/98	15.56	1.11	0.00
1997-98	04/18/98	18.33	5.56	0.00
1997-98	04/19/98	15.56	1.11	0.00
1997-98	04/20/98	15.56	1.11	0.00
1997-98	04/21/98	22.78	2.78	0.00
1997-98	04/22/98	21.11	7.78	0.00
1997-98	04/23/98	21.67	10.56	0.45
1997-98	04/24/98	13.33	3.89	0.14
1997-98	04/25/98	15.00	0.56	0.11
1997-98	04/26/98	15.56	1.11	0.00
1997-98	04/27/98	18.89	4.44	0.00
1997-98	04/28/98	22.78	5.56	0.00
1997-98	04/29/98	25.56	7.78	0.00
1997-98	04/30/98	28.33	8.89	0.00
1997-98	05/01/98	26.67	9.44	0.00
1997-98	05/02/98	27.78	9.44	0.39
1997-98	05/03/98	21.11	8.33	0.93
1997-98	05/04/98	21.11	11.11	0.00
1997-98	05/05/98	21.11	11.11	0.00
1997-98	05/06/98	20.56	11.11	0.00
1997-98	05/07/98	22.78	8.33	0.00
1997-98	05/08/98	18.89	10.00	0.03
1997-98	05/09/98	13.33	5.56	0.18
1997-98	05/10/98	11.67	7.22	0.06
1997-98	05/11/98	14.44	7.78	0.00
1997-98	05/12/98	13.89	8.33	0.02
1997-98	05/13/98	11.67	8.33	0.69
1997-98	05/14/98	12.22	7.22	0.39
1997-98	05/15/98	13.89	5.56	0.36

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	05/16/98	12.78	7.22	0.34
1997-98	05/17/98	12.22	6.67	0.32
1997-98	05/18/98	15.56	3.89	0.08
1997-98	05/19/98	18.89	7.22	0.62
1997-98	05/20/98	17.22	9.44	0.33
1997-98	05/21/98	14.44	8.33	0.00
1997-98	05/22/98	16.67	2.22	0.02
1997-98	05/23/98	16.67	8.89	0.00
1997-98	05/24/98	18.33	11.67	0.20
1997-98	05/25/98	15.56	6.11	0.33
1997-98	05/26/98	10.00	3.89	0.50
1997-98	05/27/98	16.11	2.22	0.29
1997-98	05/28/98	18.89	7.22	0.00
1997-98	05/29/98	23.33	11.11	0.45
1997-98	05/30/98	12.78	7.22	0.70
1997-98	05/31/98	18.89	8.33	0.00
1997-98	06/01/98	25.56	7.22	0.00
1997-98	06/02/98	23.89	6.67	0.00
1997-98	06/03/98	19.44	5.00	0.00
1997-98	06/04/98	23.33	10.56	0.00
1997-98	06/05/98	17.78	8.33	0.00
1997-98	06/06/98	22.78	10.00	0.00
1997-98	06/07/98	26.11	10.56	0.00
1997-98	06/08/98	25.00	10.00	0.00
1997-98	06/09/98	23.33	8.33	0.00
1997-98	06/10/98	26.11	12.22	0.40
1997-98	06/11/98	19.44	8.89	0.00
1997-98	06/12/98	21.67	11.67	0.00
1997-98	06/13/98	25.00	8.33	0.00
1997-98	06/14/98	22.22	5.56	0.00
1997-98	06/15/98	23.89	11.11	0.00
1997-98	06/16/98	20.56	9.44	0.00
1997-98	06/17/98	21.11	6.67	0.00
1997-98	06/18/98	25.00	4.44	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	06/19/98	18.33	8.33	0.00
1997-98	06/20/98	21.11	8.89	0.00
1997-98	06/21/98	26.11	9.44	0.00
1997-98	06/22/98	27.78	10.56	0.00
1997-98	06/23/98	21.67	10.00	0.00
1997-98	06/24/98	23.33	11.11	0.39
1997-98	06/25/98	20.00	11.11	0.36
1997-98	06/26/98	16.67	11.67	0.26
1997-98	06/27/98	21.67	7.22	0.04
1997-98	06/28/98	24.44	9.44	0.00
1997-98	06/29/98	27.78	8.89	0.00
1997-98	06/30/98	25.56	11.11	0.00
1997-98	07/01/98	21.11	12.78	0.00
1997-98	07/02/98	21.11	13.33	0.00
1997-98	07/03/98	24.44	12.22	0.00
1997-98	07/04/98	19.44	13.89	0.00
1997-98	07/05/98	21.67	12.78	0.00
1997-98	07/06/98	25.56	13.89	0.00
1997-98	07/07/98	29.44	12.78	0.00
1997-98	07/08/98	27.22	10.56	0.00
1997-98	07/09/98	28.33	12.78	0.00
1997-98	07/10/98	29.44	11.67	0.08
1997-98	07/11/98	24.44	9.44	0.02
1997-98	07/12/98	27.22	11.11	0.00
1997-98	07/13/98	25.56	9.44	0.00
1997-98	07/14/98	27.78	11.67	0.00
1997-98	07/15/98	28.33	11.11	0.00
1997-98	07/16/98	31.11	11.67	0.00
1997-98	07/17/98	35.00	10.56	0.00
1997-98	07/18/98	32.22	11.67	0.00
1997-98	07/19/98	28.89	11.11	0.00
1997-98	07/20/98	26.67	12.22	0.00
1997-98	07/21/98	30.00	11.67	0.00
1997-98	07/22/98	33.33	12.22	0.00

Growing season	Date	Air Temperature ^{0}C		Precipitation (cm)
		Maximum	Minimum	
1997-98	07/23/98	33.89	11.67	0.00
1997-98	07/24/98	30.56	11.67	0.00
1997-98	07/25/98	28.89	10.56	0.00
1997-98	07/26/98	30.56	16.11	0.00
1997-98	07/27/98	37.78	16.67	0.00
1997-98	07/28/98	39.44	15.00	0.00
1997-98	07/29/98	37.22	14.44	0.00
1997-98	07/30/98	27.78	10.56	0.06
1997-98	07/31/98	26.67	15.00	0.00
1997-98	08/01/98	23.89	14.44	0.00
1997-98	08/02/98	26.67	12.22	0.00
1997-98	08/03/98	32.22	10.00	0.00
1997-98	08/04/98	34.44	13.89	0.00